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THE COMPOUND OOSPHERE OF ALBUGO BLITI.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY. XVI.

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(WITH PLATES XI–XV)

THE results of this investigation introduce factors entirely new in their essential features and at variance with the cytological processes connected with fertilization previously described for coenocytic or other forms of plants or animals. The title of the paper must suggest a contradiction of terms. The writer hesitated before using the expression compound oosphere, but it seemed better, at least for the present, to modify the term oosphere which has become firmly fixed in descriptive botany so that it would express the peculiar conditions here set forth. If future investigations should indicate that the peculiarities of the compound oosphere are not exceptional, it may be necessary to introduce a new term indicating the conditions and the process of fertilization presented in this paper.

A compound oosphere is one containing several or many functional sexual nuclei. This idea violates the present conception of the structure of that cell as it exists throughout the plant kingdom, and furthermore, so far as the writer is able to judge, the

ova of animals present no such peculiarity. If there is any character that defines the oosphere and ovum it appears to be the presence in the cytoplasm of a single female nucleus that is normally fertilized by a single male nucleus. In *Albugo Bliti* Biv., however, the mature oosphere contains many female nuclei, and fertilization is effected by the discharge of many male nuclei from the antheridial tube and their subsequent fusion with the female nuclei in pairs. An oospore results from this multiple sexual act with about one hundred fusion nuclei, which remain in the resting condition until germination. The existence of such conditions must be supported by strong evidence, and great caution should be exercised in interpreting the data upon which the conclusions are based.

One is partially prepared, however, for the acceptance of such conditions as these by the thought that the form under consideration is a coenocyte, and that comparatively little is known of the behavior of the nuclei and cytoplasm in such structures. Excellent summaries of current knowledge are given by Humphrey ('92), Zimmerman ('96), and Wager ('96). It is unnecessary, in view of the existence of these accounts, to enter into details here. Suffice it to say that in several (Monoblepharidinaceæ, Entomophthoraceæ, and Chytridineæ) of the many coenocytic groups the behavior of the nuclei in fertilization is practically unknown. In those groups of which there is more knowledge (Saprolegniaceæ, Peronosporeæ, Zygomycetes, and Siphoneæ) concordant results have not yet been attained. For example, in the Saprolegniaceæ the question is still in dispute whether or not fertilization occurs (Hartog '95, Trow '95). In the Siphoneæ the two most comprehensive papers (Behrens '90, Oltmanns '95) upon the one genus (Vaucheria) that has been investigated disagree essentially as to the events leading to the development of the oosphere. The process of fertilization described for the Zygomycetes by Léger ('95) involves the unique phenomenon of the fusion of nuclear complexes.

The pioneer work on the histology of the Peronosporeæ, done by Wager on *Peronospora parasitica* in 1890, was followed by an

article by the same author in 1896 on *Albugo candidus*. Berlese in 1898 published an article on the entire group. It appears that most of the study of the histology of coenocytic fungi has been concerned with the Peronosporeæ, but even here the data are yet too scanty to admit of any wide generalizations. The improvements in technique during recent years should be held in mind, as much of the discrepancy between the earlier and later results may be thus explained.

Wager's work (**'96**) should be consulted for a comprehensive summary of the knowledge up to the time when he published the results of his investigations. For *Albugo candidus* his own research shows a condition where the antheridial tube liberates one sperm nucleus which fuses with the solitary female nucleus in the ooplasm. He employed corrosive sublimate in saturated aqueous solution as a fixing agent, and stained the sections with Hartog's nigrosin-carmine. In his earlier work on Peronospora Wager describes a multinucleate oogonium and antheridium. The nuclei of the maturing oogonium pass to the periphery, where they divide mitotically. Two or three then return to the center and probably fuse, as only one nucleus is found there at a later stage. The antheridial nuclei divide simultaneously with those of the oogonium. The antheridial tube contains one or more nuclei, the antheridium finally having many less than it had at an earlier stage. This material was fixed by either absolute alcohol or chromic acid, and stained with Kleinenberg's hæmatoxylin.

Berlese gives four figures to illustrate the development and fertilization of *A. Portulacæ*, but his description of the nuclear transformation is not illustrated. All statements concerning Albugo seem to have been based on either this species or herbarium specimens of other species. He studied also four species of Peronospora. For a killing agent he used either 95 per cent. alcohol, alcoholic corrosive sublimate, Flemming's solution, or picric acid, and stained with Flemming's triple stain or Hartog's nigrosin-carmine. Further reference will be made to these articles later in the paper.

The results presented in this paper were obtained mainly

from material fixed in chrom-acetic acid, cut in serial sections in paraffine, and stained on the slide by Flemming's triple stain. For full details regarding methods the reader is referred to the end of the paper. This investigation was begun in 1897, one year being spent in the botanical laboratory of the Ohio State University through the kindness of Dr. W. A. Kellerman. I am indebted to Mr. J. H. Schaffner for many courtesies during my work at the same institution. Since the summer of 1898 the study has been continued under the direction of Dr. Bradley Moore Davis in the Hull Botanical laboratory of the University of Chicago, where I have also received helpful advice and suggestions from Dr. J. M. Coulter and the members of the botanical staff. I wish to express my thanks to my wife for much kind assistance, in particular for the preparation of Plate XV.

DEVELOPMENT OF THE OOGONIUM AND ANTHERIDIUM.

The character of the mycelium of Albugo varies with the nature of the host tissue. The hyphae are slender where the cells of the host are thick-walled and placed close together, while in loose tissue they may swell to a considerable diameter. *Fig. 42* shows the general structure of the hyphae, the roundish nuclei, each with a prominent nucleolus and membrane, being distributed irregularly through the vacuolate cytoplasm. A single nucleus is represented in *fig. 1*. It is worthy of attention principally because of its very faint linin network. It has an actual diameter of from 2–2.5 μ.

The oogonium may be terminal or intercalary, its walls being simply the greatly expanded mycelial wall, as is evidenced by the frequent persistence of haustoria on its surface. Even very early stages of developing oogonia may be distinguished from enlarged mycelia by certain peculiarities of the protoplasm (*fig. 43*.) The nuclei are elongated, the vacuoles are angular and distorted, and the cytoplasm is drawn into stringy bands; all of which gives evidence of a disturbance not present in the ordinary mycelium. These peculiarities are frequently evident in vegetative hyphae a distance of 200 μ from the developing oogonium.

Similar appearances were noted by Istvanffi ('95) and Wager ('96), and their explanation is undoubtedly the true one, namely, that the protoplasm was rapidly flowing from the mycelium to fill the enlarging oogonium. When sufficient nucleated protoplasm has entered the developing oogonium, this structure is cut off from the hyphae by a septum at the point of enlargement (*fig. 44*). The oogonium is now fully differentiated from the vegetative hyphae, the nuclei recover their original form and lie in a coarsely vacuolate and somewhat granular cytoplasm. The general appearance of the oogonium and its contents may be seen in *fig. 45*.

The antheridium develops simultaneously with the oogonium, but gives no evidence of the flowing of the protoplasm into the growing structure. Probably owing to the small size of this organ there is but little disturbance as it fills with protoplasm. It becomes cut off from the parent hypha and the contents are similar in appearance to those of the oogonium as is shown in *fig. 45*.

The most conspicuous feature in this early development of the oogonium is the increase in the size of the nuclei. This seems to occur somewhat rapidly just before the oogonium has attained its full size. As the nuclei grow larger the linin network becomes much more prominent, until finally it assumes a very characteristic structure in the form of large meshes, the threads being coarse, densely staining, and apparently homogeneous in structure. The whole appears to be a connected network that lies against the nuclear membrane. When the threads are seen in transverse view they appear as round or oblong bodies about the size of the nucleoli, but staining differently. This condition of the nucleus, which is shown in *fig. 3*, may be regarded as the spirem stage of early mitosis. While the nuclei are passing into this condition the oogonium wall thickens slightly.

It is at this time that the number of nuclei may be determined most easily and accurately. The count ranged from 226 to 333, the smaller number being quite exceptional. Making allowance for the fact that several nuclei may readily have been

counted twice in adjacent sections, it would perhaps be fair to place the average at about 250. Wager found 115 in *A. candidus*, and Berlese 200 in *A. Portulacæ*. It is very difficult to make an accurate count in the antheridium, because this structure is so small and of such irregular shape that it is impossible to recognize its limits in adjacent sections. However, an average based on several counts indicates that the number is likely to be about 35. This number is considerably greater than that suggested by either Wager or Berlese, both of whom report about 12 nuclei in the antheridium.

When the oogonial nuclei are passing into the spirem condition the *Hautschicht* seems to be closely appressed to the wall in the vicinity of the antheridium. This fact is demonstrated most clearly in preparations where there has been slight collapse of the contents of the oogonium (*figs. 47, 48*) and the protoplasm has shrunken away from the wall everywhere excepting at the point opposite the antheridium. This adherence of the *Hautschicht* is correlated with a very marked granulation of the cytoplasm in this region, a phenomenon also noted by Wager, and one which seems to be significant. It suggests that a cellulose enzyme is secreted to dissolve the wall of the oogonium. As indicated in *figs. 48, 49, 50*, this wall frequently shows the marks of corrosion over a considerable area, always at a point opposite the antheridium. This interesting process results in a neat perforation, through which the cytoplasm of the oogonium flows so as to form a very conspicuous swollen papilla within the antheridium. Various stages in this process are shown in *figs. 50–55*. It is difficult to explain this phenomenon. The initiatory step in the perforation of the oogonium wall seems without doubt to be taken by the protoplasm of the oogonium itself. But what is the significance of the pushing of the cytoplasm of the oogonium into the antheridium to form the peculiar bubble-like papilla? The structure, both wall and contents, stains deeply, thus becoming very conspicuous, while its extremely frequent occurrence, as well as its presence in other species, seems to indicate that it is not abnormal. The papilla wall is so extremely thin that one

may only conjecture whether or not it is derived from the antheridium wall. The history of the papilla beyond the stage shown in *fig. 54* is not clear. In this condition the structure is very thin-walled and its contents vacuolate, resembling a large irregular compound bubble. Whether the delicate wall now bursts or the contents are gradually withdrawn into the oogonium is uncertain. At all events, the papilla in later stages leaves no trace of its former existence. There follows at a later period conditions (*figs. 56, 57*) which show that there is certainly a movement of the cytoplasm in the opposite direction, the antheridium extending a process with a cell wall through the opening into the oogonium. The first surge of the cytoplasm from the oogonium into the antheridium may be due simply to the unequal conditions of turgor in the two structures, but it is possible that there is also a phylogenetic significance in the phenomenon. The occurrence of similar structures in *A. candidus* and *A. Portulacæ* shows it to be of some import. Such a papilla in a much less highly developed form is figured by Wager and referred to as the receptive papilla.

The antheridial tube presses into the oogonium in the form of a slender thin-walled process (*figs. 56, 57*). It is filled with dense cytoplasm that greedily absorbs and retains stain, and is surrounded by a sheath of dense oogonial cytoplasm. The nuclei remain in the antheridium, none entering the tube at this time, and are indistinguishable in size and structure from those in the oogonium. They are also in the spirem condition, similar to that described for the oogonial nuclei. The description of the further development of the antheridial tube is deferred, to follow the account of the differentiation of the compound oosphere.

DIFFERENTIATION OF THE COMPOUND OOSPHERE.

The previous description carries the history of the sex organs up to a time when the antheridial tube has penetrated the oogonium one fourth or one fifth the diameter of that structure. Correlated with the further development of the tube there occurs the differentiation of the periplasm and ooplasm, and the

extrusion of the nuclei from the central region of the oogonium. The process consists essentially in a centripetal movement of the cytoplasm, and results in a massing of this cytoplasm in the center of the oogonium in such a manner that the vacuoles and nuclei are carried to the periphery of the denser central portion thus developed. Behrens ('90, 315) describes a somewhat similar condition in Vaucheria as follows: "Der ganze Vorgang besteht also in der Ablösung des grössten Theils der Protoplasten von der Wand durch Vacuolisation der wandständigen Plasmaschichten." This curious phenomenon was noted by Wager in *A. candidus*, and subsequently by Berlese in *A. Portulacæ*. The process as heretofore described is simple. In *A. Bliti* it is complex, but unique and full of interest; and as a complete knowledge is essential to an understanding of the further development of the oosphere a detailed description must be given.

The first hint of the centripetal aggregation is found in a tendency of the cytoplasm to depart from the even distribution shown by a young oogonium, and to collect in masses throughout the interior (*fig. 58*). These denser portions run together, forming fewer but larger masses (*fig. 59*). Thus several prominent aggregations of cytoplasm may be formed, separated from one another and from the wall by vacuoles of varying sizes (*fig. 60*). These denser regions are homogeneous in structure, containing minute vacuoles of uniform size evenly distributed in a matrix of cytoplasm free from granules. The dense regions contain no nuclei, because these are forced from the dense cytoplasm to a position on its periphery. The dense centers now coalesce, forcing out the vacuoles. This may result immediately in the condition shown in *fig. 61*, but frequently the coalescence proceeds more slowly and irregularly, and often a reniform mass is formed, the indentation on one side marking the juncture yet to be made. The last gap narrows until only a few vacuoles remain to mark its track (*fig. 61*), and these soon float outward leaving one mass of cytoplasm, the rudimentary oosphere (*figs. 62, 64, 65*). As the vacuoles pass outward they often leave captive nuclei in their wake (*figs. 61, 62, 64*), but these soon follow. A typical view

of the resulting condition is presented in *fig. 64*. The outer region of the oogonium, the rudimentary periplasm, is coarsely vacuolate, presenting a conspicuous contrast to the dense central mass. Along the boundary between these two regions are gathered most of the nuclei (*fig. 64*).

The next stage in the differentiation of the oosphere is conspicuous and clearly characterized. It ends in producing a distinct differentiation between the oosphere and the periplasm (*fig. 65*). This condition is brought about primarily by the marshaling of the nuclei into an oval or an irregular hollow sphere, a section of which is shown in *fig. 65*, while a somewhat earlier stage is to be seen in *fig. 64*. Both figures illustrate the one important fact that all or nearly all of the nuclei are at the boundary of the central dense mass. The latter figure in addition shows that there is a sharp line of demarcation between the ooplasm and periplasm. There are usually a few scattered nuclei in the periplasm, and occasionally one finds a nucleus in the oosphere that has not passed out as rapidly as the others.

Important changes occur in the cytoplasm while the nuclei are arranging themselves into a hollow sphere. At the beginning of this process the region that is to become periplasm is coarsely vacuolate, in marked contrast to the dense cytoplasm of the center, but the two regions blend gradually together where they meet (*figs. 62, 64*). Later when the hollow sphere of nuclei becomes more regular in outline dense granular cytoplasm is differentiated around and between the nuclei (*figs. 65, 68, 69*). The inner border of the rudimentary periplasm also becomes differentiated into a film more densely granulated than any other region of the oogonium, and finally determines the limit of the oosphere. There is not yet an organized wall, and the most critical study reveals nothing more than a dense film of protoplasm. It is convenient to call this condition the stage of zonation.

The position of the stage of zonation in the sequence of events leading to the differentiation of the oosphere is clear. With this condition comes the characteristic and sharp limitation

between ooplasm and periplasm which is maintained until maturity, while before zonation such a differentiation did not exist. The process of differentiation is gradual, and a series of developmental stages has been obtained which seems complete. This period is the only one where the ooplasm possesses very few nuclei or none, and it is impossible to regard it as being a period later than stages which present zonation and also contain 50–100 nuclei (*figs. 68, 69, 70*). The development of the antheridial tube is such as to lend strongest support to the sequence above indicated, since the tube is shorter in stages preceding zonation and longer in stages following it (see plates), thus affording strong corroborative evidence. While the differentiating line is characteristic of zonation the paucity of nuclei in the ooplasm is equally so. The sharper the differentiation the fewer the nuclei, and when zonation was very definite none could be found, and it is probable that when this stage is at its highest development there are no nuclei in the ooplasm. There is some evidence, however, that makes it seem possible that one and even two spindles sometimes remain in the ooplasm, but this is uncertain.

No mention has been made, as yet, of the division of the nuclei of the oogonium. This mitosis closely accompanies the process of zonation as is indicated in most of the figures. These two events apparently take place nearly simultaneously. The earliest prophase is typical in such stages as are shown in *figs. 58, 59, 60, 61*. At the time of complete zonation the nuclei are in metaphase and lie close to the line that separates the ooplasm from the periplasm (*figs. 65, 66*). Spindles are frequently found that actually cross this line at right angles, so that one pole lies in the ooplasm and the other in the periplasm (*figs. 65, 66*). The mitoses that take place at this period mark an important and characteristic phase in the history of the oogonium. Those dividing nuclei that lie tangential to or wholly outside of the boundary line between the ooplasm and periplasm leave their daughter nuclei in the periplasm. Each of the spindles which cross the line (*fig. 66*) gives one daughter nucleus to the oosphere

and the other to the periplasm, and the line of differentiation is sharply defined and unmistakable. Nuclei may be observed in every phase of this mitosis, and the daughter nuclei may be found in all stages of reorganization, one of each pair in the ooplasm the other in the periplasm. The writer was unable to detect any difference between the mitoses that occur strictly in the periplasm and those that contribute daughter nuclei to the oosphere. As a result of the division a large number of nuclei pass into the ooplasm, thus producing a multinucleate cell containing by actual count an average of 45 to 55 nuclei (not less than 40 or more than 60). The oosphere is thus a coenocyte; instead of the uninucleate cell which one would expect there is found a multinucleate structure, to designate which the writer has used the term compound oosphere.

Because of the importance of the anomalous compound oosphere and the peculiarities of its development, it seems best to discuss at length difficulties that might be suggested. It may be claimed that a mistake has been made in the sequence of events, and that the multinucleate condition of the central region does not follow, but precedes zonation. This objection is invalid, for four reasons: (1) the sequence is complete up to zonation, and there is no place for a multinucleate central region in the series; (2) the studies in cytology make it certain that there is but one simultaneous mitosis of the oogonial nuclei, so that the phase of the division serves as an index of the age of the oogonium, thus rendering any misunderstanding of the sequence impossible; (3) the interpretation presented is necessary to the understanding of stages that are positively and unmistakably older; (4) the development of the antheridial tube and central body correlate with the views presented. It would seem impossible that a mistake in seriation has been made. It may be claimed that the daughter nuclei seen in the mature oosphere are not *in situ*, but were carried in by the knife. There is abundant evidence to controvert this claim, for the nuclei in anaphase often lie in such positions that, if carried in by the knife, they must have been carried in two opposite directions by the same

stroke. It may be well to state that specimens of every structure or stage represented or described in this article are preserved, not in one, but in several mounts, and for most of the important stages in many preparations. No isolated or single fact is anywhere used either to support or destroy any theory.

With the entrance of the nuclei differentiation of the oosphere is complete. The ooplasm is of very fine, even texture, made up of such small meshes that the vacuoles are never more than half the size of the nuclei, and there are no prominent granules or oil drops shown by the Flemming stains at this time (*figs. 68–69*). The periplasm is loosely vacuolate, the strands are often granular, and the nuclei are frequently at their intersections, often in bunches.

The nuclei of the antheridium usually divide simultaneously with those of the oogonium, this being so constantly true that from a glimpse of one organ the condition of the other could be predicted. No difference between mitosis in the oogonium and in the antheridium could be observed. At the time of the differentiation of the oosphere the contents of the antheridial tube stain deeply, but it has not been possible to demonstrate nuclei in its interior at this age. When the nuclei line up preparatory to zonation the antheridial tube has usually penetrated the periplasm almost to the outer boundary of the oosphere, and as it later pushes into the oosphere, during the telophase of the mitosis, it somewhat indents the boundary film. One oogonium was seen that had two antheridial tubes penetrating the periplasm from opposite sides, but neither had yet pierced the boundary of the oosphere. Another stage was observed where an unemptied tube lay tangential to the oosphere, being apparently the unfavored of two competitors. Still another case presented a stage of fertilization in which two tubes were simultaneously opening into one oosphere.

Minute globules of brownish color are to be seen in the cytoplasm of the oogonium from its earliest development up to zonation. In all early stages they are indefinite in number and irregular in size, but seldom larger than one of the nuclei.

When the ooplasm is first differentiated it possesses a small number of these globules which are irregularly distributed. At this time and in earlier conditions there is nothing to indicate that these structures have any peculiar significance. In their color and form they suggest minute drops of some oil-like substance that has remained undissolved under all the treatment experienced by the preparation. However, at the time of zonation, and in certain later stages, there is only one such drop or globule, where there were previously several, and that one is always in the geometrical center of the oosphere, and is surrounded by a differentiated region of cytoplasm (*figs. 69–71, 74*). Whether or not this one central globule, which is a constant feature of the oosphere from zonation until just before fertilization, is developed by a fusion of the drops previously present could not be determined with certainty. This is strongly suggested, however, by appearances like those noted in *fig. 75*, which was drawn from a young oosphere, and seems to indicate that several minute drops were fusing to form the central globule.

As the oosphere matures the central globule remains unchanged, its constancy in size being very remarkable; but the cytoplasm immediately surrounding it becomes more dense, although still shading away gradually toward the outside (*figs. 41, 69, 71, 74*). In all stages observed this globule was surrounded by the remarkable region of dense cytoplasm which differs from ordinary ooplasm in that it stains more darkly and contains fewer vacuoles and nuclei. When the whole central structure is at its maximum development it is a very conspicuous object within the oosphere (*figs. 41, 71*). There occurs also further slight differentiation of the cytoplasm in the immediate vicinity of the globule where the stain is taken more faintly or has a yellowish tint. This inner region also shows a radiate structure under the low power (*fig. 69*), but the highest magnification, such as that employed in drawing *fig. 71* (3300 diameters), failed to demonstrate definite fibers. *Fig. 71* shows in detail this peculiar region of the oosphere; outside is the vacuolate ooplasm, then comes the region of dense cytoplasm with few vacuoles, and finally in the

center is the opaque globule immediately surrounded by a lightly stained zone. After reaching the condition of maximum development the structure rapidly loses character, disappearing entirely just before the antheridial tube discharges its contents.

A summary of the history of the central structure may be given as follows: it first appears in zonation, and reaches its maximum development when the daughter nuclei of the first mitosis pass into the oosphere; after that it rapidly degenerates, although traces of its presence sometimes persist nearly to the time of fertilization. A body of apparently similar nature was mentioned by Wager as occurring in the oosphere of *A. candidus*, and my own as yet incomplete observations on that species indicate that the body seen in *A. candidus* and the central globule of *A. Bliti* are homologous structures, although they differ much in certain details. I find also a structure very like the one above described in the oosphere of *A. Tragopogonis* and *A. Portulacæ*, and believe that we have here an organ of the oosphere, perhaps regularly present in the whole genus Albugo, if not in the oospheres of other related genera. It appears with such constancy at certain important stages in the life history of the species, and passes through such a definite course of development that its presence seems to be of importance. May it not be an organ of the coenocytic oosphere?[1]

It should be called to mind in this connection that Dangeard noted in each of the numerous oospheres, in certain species of the Saprolegniaceæ and Peronsporeæ, a central body which appeared just before fertilization. Various interpretations have been given to such structures by different writers, early observers mistaking it for a nucleus. Dangeard supposed it to be oil, but Wager thought that Dangeard was probably mistaken, and that the structure is truly a central body such as he himself found in *A. candidus*. The descriptions and time of appearance make it seem quite possible that the body noted in the Sapro-

[1] MR. SWINGLE expressed such views at the meeting of the Society for Plant Morphology and Physiology at Ithaca, December 1897.

legniaceæ may be homologized with the central globule of the oosphere of Albugo.

It is premature to discuss the function of this body until its relations in other species have been closely studied. As described by Wager it appears to be intimately connected with the behavior of the sexual nuclei; but there appears to be no such relation in *A. Bliti*, where its function is perhaps that of an organizer in the oosphere. The body first appears when the oosphere is well defined and is most highly differentiated during the entrance of the daughter nuclei. At this time also occurs the formation of a thin film of denser protoplasm which definitely bounds the oosphere. A great dynamic change occurs in the oogonium when the ooplasm and periplasm are differentiated and the zone of cytoplasm separating the two regions is formed, and in addition there is that remarkable division of the nuclei in such a manner that approximately fifty daughter nuclei are always cast into the ooplasm. Simultaneous with these activities, existing when they are at their maximum and disappearing when they cease, there is formed this peculiar structure, which is so definite in character and so constantly present that it seems to have some functional importance.

In view of the morphological character and possible physiological value of this central structure to the coenocytic oosphere the writer ventures to propose for it the name *cœnocentrum*. It is necessary here to emphasize the difference in structure between the cœnocentrum and a nucleus. The globule is distinctly not a nucleus, as it is much smaller than any nuclei that were seen, stains differently, and is structureless and unchanging. The cœnocentrum cannot be a nucleus with the globule as a nucleolus, since these masses fail to have the internal structure of a nucleus or its limiting membrane. There is no definite demarcation from the surrounding ooplasm, as is plainly shown in *figs. 41, 71, 74, 75*.

In *fig. 75* other structures are shown, the nature of which is unknown. They are small granules which lie profusely distributed in the cytoplasmic strands in all ages of the oogonium, but

are particularly noticeable in the fine meshed area of the oosphere. They do not appear when stained in the ordinary way by Flemming's triple stain, but seem always present and conspicuous if stained by Heidenhain's hæmatoxylin, when they are black (*fig. 75*). A hæmatoxylin preparation when bleached and restained for a long time in safranin shows numerous round red bodies in the same position. In appearance they are a trifle longer than broad and often double, as though two were lying end to end, reminding one of large bacilli. It does not seem probable that these coalesce to form the central dot, as might be suggested by *fig. 75*, since they have a different reaction to stain.

SIMULTANEOUS MITOSES IN THE OOGONIUM.

During the differentiation of the oosphere the nuclei in the oogonium divide once (*figs. 58–62, 65–67*), the mitosis occurring about simultaneously for all of the nuclei, cases of independent division of single nuclei never being found. Almost invariably an oogonium in the condition of zonation presents the nuclei in metaphase, or just passing into anaphase (*fig. 65*). In earlier stages, just before zonation, when the cytoplasm is massed in one or several centers, the nuclei are usually in prophase (*figs. 59–62*), but metaphase may be present even as early as the beginning of this process (*fig. 58*). It is apparent that anaphase is never reached until after zonation, and that the mitoses begin when the cytoplasm commences to collect in masses. This nuclear division always occurs when the antheridial tube is in the position shown in *fig. 59*. It would seem, *a priori*, that the differentiation of the oosphere would take place rapidly, since it consists merely in a floating out of the vacuoles and nuclei from the interior region of the oogonium, but mitosis is presumably less rapid. If changed conditions should hasten or retard the process of zonation, one would expect a variation such as does exist in the time correlations between zonation and the mitoses.

The spirem condition of the nucleus has been described, but the other stages of nuclear division have not been considered in

detail. Preparatory to the formation of the spindle the nucleus elongates, its membrane being pulled out in two directions, while the chromatin collects in minute globules in the linin thread. These are at first irregular in size, but gradually become less numerous and more uniform, presumably fusing with one another until a small number of nearly spherical bodies, the chromosomes, are present in the nucleus. *Fig. 4* shows a condition with the chromatin granules and the linin network still evident, while in *fig. 5* the linin strands have almost disappeared. As the chromosomes perfect their organization they approach the equator of the now elongated nucleus (*fig. 6*), and there appear at the poles two round bodies which lie within the nuclear membrane. These bodies stain red with Flemming's triple stain and are constantly present at this period of the prophase. Although not observed earlier they persist and become more prominent in the later stages. The spindle fibers first appear a little later at the poles of the elongated nucleus, from whence they seem to grow toward the equator. They are entirely intranuclear, and there is a distinct space between them and the nuclear membrane (*fig. 9*). The chromosomes are at first irregularly scattered throughout the equatorial region, but when the achromatic figure becomes more distinctly developed they group themselves into a nuclear plate and divide. As the chromosomes are nearly spherical and very small it was impossible to determine the manner of their division. The mature spindle rests in a clear region, distinctly inside of the nuclear membrane, with the polar bodies very definitely outlined. That these bodies must be regarded as centrosomes is evident from their constancy at certain periods of the mitosis, *e. g.*, from late prophase to late anaphase. Being intranuclear, it is not surprising that extranuclear radiations should be absent, and in fact the only radiations present are the spindle fibers. These structures seem not to have been previously described for this group of fungi.

Figs. 7, 8 show a condition very commonly seen. The nuclear membrane is prominent, the chromosomes are massed at the center, and the spindle fibers are very slightly or not at all

differentiated. The dark strand shown in the lower half of *fig. 8* is probably like the less conspicuous one in a similar position in *fig. 5*. Both may be considered as the remains of a spirem thread, such as is shown in *fig. 4*. *Fig. 7* is open to a similar explanation. The difference in the shape of these two figures is noteworthy, since it is probably due to their position in the oogonium. The spindle shown in *fig. 8* lay in a strand of periplasm which supported an oosphere, similar to the strands shown in *fig. 62*. It is probable that the length of the spindle is due to the tension to which it was subjected. *Fig. 7* was from a crowded bunch of nuclei, and could not elongate. The appearance shown in this figure might tend to support the idea that the spindle fibers are formed from the linin thread, a view entertained by Wager, but disputed by Berlese. The question presents so many difficulties that the writer does not feel warranted in expressing an opinion.

The nucleolus at the time of late prophase is sometimes small, but often quite as large as when the nucleus is in the spirem condition. It may be found throughout all stages of the mitoses. *Fig. 12* shows the splitting of the chromosomes, and *fig. 13* may be recognized as a condition immediately later; the membrane is still intact and encloses the nucleolus which lies outside of the spindle, and the centrosomes are at their maximum definiteness. It is interesting to note in passing that the few nuclei lying very near to the antheridial tube are usually nearly a full phase in advance of other oogonial nuclei in mitoses, a fact strikingly apparent when the majority of the nuclei are in metaphase.

The chromosomes, after the division of the nuclear plate, move poleward with unequal rapidity, the poles lose their acute character, and the nuclear membrane is no longer visible, the boundary of the nucleus being marked by the spindle fibers (*fig. 14*). With the loss of the membrane the whole nuclear structure assumes, and retains through later stages, the property of staining more darkly, a character particularly noticeable in the regions where the chromosomes lie (*figs. 14–18*).

The nucleolus may travel poleward with one group of chromosomes, or break into two, either at this time or earlier, thus allowing a small nucleolus to go to either pole. It is easily distinguished from the chromosomes by stain reaction and usually also by its size. At this stage there is at each apex a round body of the size and shape of the centrosomes, but scarcely dis tinguishable from the chromosomes except through position. Even in very late anaphase faint fibers may be seen connecting the daughter nuclei (*figs. 15, 16*). When the chromosomes reach the end of the spindle they become indistinguishably mingled and massed (*fig. 16*), but the nucleolus often stands out very distinctly by virtue of its color and size.

After the two groups of chromosomes are sufficiently separated the spindle fibers collapse in the middle (*fig. 18*), and the daughter nuclei become distinctly organized. Each rounds off and contains a dark somewhat crescent-shaped mass of chromatin on the side that is turned away from its sister nucleus. This condition is often very noticeable in the differentiated oosphere when several daughter nuclei may be observed, each with its dark half centerward. The explanation of this condition is not far to seek. The sister nuclei lie in the periplasm with their dark half turned outward, plainly showing that the former mitotic figure lay across the line that separated the ooplasm from the periplasm. These conditions present strong evidence of the source of the nuclei in the oosphere.

Whether or not the centrosome of Albugo persists as a permanent organ of the cell is a question that as yet is impossible to answer. The structure so prominent at metaphase is not seen in the resting nucleus; the conditions, however, are such that it might well exist hidden among the chromatin granules and pass unnoticed. It is so small and its stain reaction so uncertain that negative evidence is valueless.

The shape of the spindle figure may be greatly modified by the conditions; for example, if the nuclei happen to be in prophase or metaphase when the centripetal rush of cytoplasm occurs the tension due to the movement of the protoplasm seems

to act conjointly with the normal elongating forces, thus producing extraordinarily long spindles (*fig. 62*). On the contrary, if the nuclei reach the border of the central mass in an earlier stage of mitosis no such forces obtain. Spindles caught in the first massing of the cytoplasm are often distorted and bent like the letter *f*, owing undoubtedly to torsion caused by the vacuoles as they move outward.

MATURATION OF THE COMPOUND OOSPHERE AND OF THE ANTHERIDIUM.

The multinucleate or compound oosphere when completely differentiated contains by actual count an average of 45–55 nuclei. These are found in various conditions of reorganization following the mitosis at zonation, and they rapidly assume the typical condition of a resting nucleus, each showing a prominent nucleolus and very faint linin network. A nuclear membrane is sharply differentiated. Presently the linin network becomes more prominent and a spirem condition is reached, very like that first observed in the oogonium. A mitosis now occurs in the oosphere affecting all of its nuclei, and is similar in all important details to that just described for the oogonium, as illustrated in *figs. 22–30*. The nuclear figure stains much more faintly than that of the previous division, the spindle appearing lighter and skeleton-like in comparison with that of the first mitoses. The only other important differences noticeable are in the more pointed anaphase and telophase figures. Compare *figs. 28–30* with *figs. 14–18*. The spindles are always long (*fig. 30*), and this fact renders it easy to detect the formation of the new membranes around the daughter nuclei by the collapsing spindle fibers. The daughter nuclei round off, pass into a resting condition, and are ready for fertilization.

While this division proceeds in the oosphere a similar mitosis occurs in the antheridium. Since the antheridial nuclei divide simultaneously with the oogonial nuclei, passing into the resting condition, and are found in mitosis when those of the oosphere divide, it is evident that they undergo two divisions. It is inter-

esting to note that the two nuclear divisions in the antheridium and in the oogonium are similar in character and proceed simultaneously. The antheridial tube at the time of the differentiation of the oosphere lies in the periplasm, with its apex close to the bounding film of the oosphere. It now pushes into the oosphere, increasing in diameter as it advances. It takes safranin stain greedily from this time until it discharges its contents, but if the stain be thoroughly extracted in acid alcohol and the preparation treated with gentian violet the contents become clear. The tube when fully developed is seen to contain numerous nuclei. A glance at *figs. 73, 76* will give a clear notion of this condition. It will be seen that many nuclei are massed near the tip of the tube and that others are apparently entering at the base. It is impossible to determine their number by actual count, owing to the crowded condition (*figs. 77, 85*, etc.). However, as there are about 35 nuclei originally present in an antheridium, and these divide twice, there must be altogether about 140. Of these, 20 or 30 perhaps remain in the antheridium proper, leaving a little more than 100 to pass into the tube. The antheridial tube pushes toward the center of the oosphere during the second mitosis (*fig. 70*), and arriving nearly at the center its tip swells, becoming nearly globular. In this condition the end of the tube is covered by a very thin wall which is barely visible, and yet holds within a dense mass of sperm nuclei (*fig. 77*).

When the male nuclei enter the antheridial tube they possess the characters of resting nuclei, but as they approach the tip they become oval, and later pointed at both ends, and the anterior end is seen to contain the nucleolus around which is massed a densely staining substance, probably chromatin. *Figs. 31, 32* shows nuclei from both the base and tip of the same tube, that which is illustrated in *fig. 73*. In the narrow entrance and basal portion of the tube the nuclei are necessarily arranged in single file, but as its diameter enlarges they become massed in dense groups, and the tip is so closely packed with nuclei that it reminds one forcibly of the appearance of a raspberry with its

drupelets (*fig. 77*). Two sections of the same antheridial tube are shown in *figs. 77, 78*, one at the tip showing numerous nuclei surrounded by a very delicate membrane, the other near the base giving a view of the narrow nearly empty cavity and the thick wall. As the tube enlarges the protoplasm in the antheridium proper becomes more and more vacuolate, but its contents never entirely leave the structure (*figs. 80a, 86*). The film separating ooplasm from periplasm is but slightly if at all changed by the entrance of the antheridial tube and during the maturation of the oosphere. The periplasm likewise shows no important changes. Some of its nuclei divide mitotically, but the number does not seem to increase materially. Most of them remain in a resting condition. One case was observed where every nucleus in the periplasm was undergoing mitotic division simultaneously with those of the oosphere, but this must be regarded as a very exceptional instance.

FERTILIZATION.

The conditions are now ripe for the act of fertilization. The female nuclei resulting from the mitosis in the oosphere, about 100 in number, are in resting condition. The antheridial tube is filled by an approximately equal number of male nuclei, and its tip has swollen so that the contents are separated from the ooplasm by only the thinnest of walls. The wall finally vanishes and the contents of the tube are free to mingle with the cytoplasm of the oosphere (*figs. 80, 82*). The sperm nuclei move through the ooplasm toward the female nuclei, their wake being often marked by a streak of denser cytoplasm. There is no visible cause of this movement, but as the male and female pronuclei differ in form a chemotropic influence may perhaps be safely inferred. Longitudinal sections of antheridial tubes (*figs. 80, 82*) sometimes show the nuclei pouring out, and transverse or oblique sections (*figs. 84, 85, 86, 77, 78*) corroborate this proof of a discharge of many nuclei. There is in all of these sections unmistakable evidence of a multinucleate discharge from the antheridium into a multinucleate oosphere. Sections of the

antheridial tube similar to those figured, both transverse and longitudinal, are not uncommon in the writer's preparations, and many have been carefully studied. No antheridial tube was found which gave any evidence of the possibility of the discharge of but one functional sperm nucleus.

When the sperm nuclei emerge from the tube their nucleoli are in the anterior ends, and later there appears prominently in the same region a substance that stains like chromatin. As the sperm nucleus approaches the female nucleus a faint linin network becomes visible (*figs. 33, 34, 35*). When the sex nuclei first come in contact the male is the smaller, but later they become approximately equal in size. It seems probable that the female nucleus actually decreases slightly in size during this equalization. The nuclei do not immediately fuse, though both are apparently in resting condition. All stages of fusion can be easily observed. The sexual nuclei are pressed together, the sperm nucleus first assuming a spherical form, the bounding membrane disappears at the point of contact, and there results one dumb-bell-shaped nucleus (*figs. 37, 38*). As the coalescence becomes more complete the fusion nucleus takes on a spherical form, and presents the structure of a resting nucleus. No details regarding the fate of the linin network were obtained, owing to the extreme minuteness of these structures and the increased difficulty in staining them in a manner adequate to their study (*figs. 35–40*). Fusion must be a process of extreme slowness, judging from the advance made in other structures of the oospore during its consummation.

A general view of an entire section of an oospore during the pairing of the sexual nuclei is shown in *fig. 88*. A count of the number of pairs in all of the sections of such an oospore gives an average of about 100. There seems to be a slight excess of sperm nuclei, as occasional small unpaired nuclei are found during the fusion stage; there are also several nuclei left in the antheridium proper and in the base of the tube. Sections of the oospore in which the nuclei are fusing present no trace of the antheridial tube inside of its wall, although it is easily traced

through the periplasm (*fig. 91*). Judging from this condition the terminal portion of the tube must vanish immediately after giving up its contents. The portion imbedded in the periplasm becomes thickened, resembling the primitive wall; but it seems never to attain the character of the mature epispore, as is the case in so many other species of Albugo. Indeed, no traces of the antheridial tube were ever seen in ripe spores.

The character of the ooplasm changes when the antheridial tube opens. As may be seen by comparing *fig. 70* with *figs. 80, 82, 84, 85* the vacuoles increase considerably in size and become irregular in form. The most striking feature of this later condition, however, is the tendency of the contents of the oosphere to break away from the periplasm (*fig. 80*), a phenomenon never met in younger stages. This indicates that changes have occurred at the boundary between the ooplasm and periplasm. Indeed, it is at this time that a true wall may be first observed around the oosphere. It will be remembered that previously the periplasm and ooplasm were separated only by the delicate film that appeared during zonation; but now for the first time a distinct wall is present around the ooplasm, and its advent seems to be correlated with the opening of the antheridial tube.

The wall occupies precisely the position of the film between the ooplasm and periplasm, and is probably formed by a further development of that structure. Its intermediate position between the ooplasm and periplasm and the apparent organic connection with both leads to the belief that it is the product of the joint action of both regions, rather than of either ooplasm or periplasm alone. Since this wall remains perfectly distinct from the walls that are formed later, it will hereafter be called the *primitive wall*. This term is used simply for convenience in this paper. Further study of related forms may establish important homologies and lead to further classification. The primitive wall is very clear and homogeneous in structure, entirely without striations, and shows great regularity of curve and thickness. From this time on the condition of the developing walls serves as an index to the age of the oospore.

A stage of somewhat frequent occurrence is shown in *fig. 87*. Judging from the presence of the primitive wall, the character of the ooplasm, and the absence of the antheridial tube, it must follow the opening of the latter, and since the nuclei are not yet paired must precede the condition shown in *fig. 88*. There are two possible explanations for this condition, consisting as it does of an oospore containing several groups of nuclei, each cluster imbedded in a mass of denser cytoplasm. Perhaps these nuclei are gathering the cytoplasm about themselves, a phenomenon of rather frequent occurrence with sexual nuclei; or it may indicate the breaking up of the mass of nuclei and cytoplasm that was released from the antheridial tube. The latter explanation seems more probable. If it be true, a stage similar to that shown in *fig. 88* would result through a further fragmentation of these nucleated masses of denser cytoplasm.

The previous pages have dealt entirely with descriptions of the antheridial tube, the discharge of its multinucleate contents, and the subsequent fusion of sexual nuclei in pairs. For the sake of completeness, and in view of the peculiarity of the conditions and the general, if not universal, belief in a simple process of fertilization, involving only two sexual nuclei, it seems desirable to discuss the possibility of such an occurrence taking place in the oosphere, together with the events already described. A simple fertilization predicates the existence of one female nucleus in the oosphere and one male nucleus in the antheridium; these are either alone, or if with others at least different in structure and function from them. Subsequent to its final differentiation the oosphere never contains less than 40 nuclei. At a later stage both antheridium and oosphere contain about 100 nuclei, but neither contains a single nucleus differing in appearance from the others. If a uninucleated oosphere exists it must be before the oosphere is fully differentiated. A glance over the drawings shows that no such uninucleate stage is represented, nor was there ever the slightest hint of such a condition found during a most persistent search, involving hundreds of oogonia. That such could have existed and escaped observation seems very

improbable. The impossibility of the central body either being or containing a nucleus has been sufficiently discussed on a previous page. The search for the single nuclei proved in vain.

The refuge left for an adherent to the idea of a simple fertilization, involving only two sexual nuclei, lies in the assumption that the nuclei of the compound oosphere (*figs. 68, 69*) have descended from a fusion nucleus, which, owing to its rapidity of development may have escaped observation in earlier conditions. That is to say, fertilization might have occurred at a stage similar to that presented in *fig. 64* or earlier. If this were true we would expect to find the ooplasm presenting 2, 4, 8, 16, 32, 64, etc., nuclei, in stages following the division of such a fusion nucleus. As a matter of fact no such conditions were ever observed, or is there the slightest evidence that they could be present. The oosphere when first differentiated contains 40–50 nuclei, derived from the mitotic figures that line up in the manner shown in *figs. 64, 65*. This number is increased to about 100 by the mitoses in the compound oosphere (*fig. 70*), and then comes the observed act of fertilization (*fig. 85*), the discharge from the antheridial tube of a large number of sperm nuclei and the subsequent fusion of these in pairs (*fig. 88*) with the female nuclei. Previous to the act of fertilization the antheridial tube gradually fills with nuclei as it presses deeper into the ooplasm. There is of course a time when the tube contains a single nucleus, but this is when it is about one third the size finally reached, and long before it shows any indication of opening.

It is true that very early in oogenesis the dense cytoplasm in the interior of the oogonium may contain a small and very variable number of nuclei, as is shown in *figs. 61, 62, 64*. But there can be no doubt that these conditions represent part of the process of zonation, and they have been discussed in that section of this paper headed "Differentiation of the compound oosphere." It is very probable that stages similar to these might be found where there is only one nucleus left behind in the oosphere in the process of zonation, but the condition of the antheridial tube and all the further history of the oosphere show that this is not the

time when an act of fertilization could possibly take place. Moreover, when only one or two nuclei are present they are *always peripheral*, which would not be expected if they resulted by the division of a fusion nucleus. Again, these scattered nuclei are always in the same condition as those near the outside of the developing oosphere, and this is almost invariably a metaphase of mitosis. This coincidence is inexplicable on the basis of their being the result of the division of a fusion nucleus, but it follows as a necessity from the explanation offered in this paper. If they are the product of one nucleus, which has undergone three or four divisions, we have to assume not only the existence of the sex nuclei, their fusion, and the fusion nucleus, but also the resting, prophase, telophase, and anaphase conditions in the formation of 2, 4, 8, 16, and 32-celled stages. An anaphase nucleus is never seen in an oogonium except when the nuclei divide simultaneously either during the first or the second mitosis. The first anaphase always occurs when the general appearance is that shown in *fig. 67*, *i. e.*, when the nuclei are completely lined up and the ooplasm is well differentiated. The second appears in the oosphere after its complete differentiation (*figs. 70, 74*). It is impossible that the 50 nuclei of the oosphere can have been derived from a single hypothetical fusion nucleus.

If attention is turned to the antheridial tube it might be suggested that fertilization could take place at an early period, when the conditions are like those shown in *figs. 62, 64*. But it is only necessary to make plain the fact that the antheridial tube is always very short at this time, and invariably occupies the position shown in *figs. 62, 64*. The wall of the tube is thick, the tube rarely if ever contains nuclei, and there is not the slightest indication that it is at all ready to open. If it be assumed, however, that it does release, in some manner difficult to detect, a single nucleus that is really the male nucleus, and which fuses with the female nucleus, how can the continued growth of the tube and the development of such peculiar conditions as are shown in *figs. 68, 70, 73, 76, 77, 80, 82, 83*, etc., be explained? Why does the tube continue to grow after functioning only to meet the difficulty

of disposing of its comparatively massive body and numerous nuclei in the ooplasm? Why do its nuclei later assume a specialized form, resembling sperms (*fig. 85*)?

In considering the positive side of the argument, in favor of a multinucleate fusion, no step is left to be filled by assumption. All of the stages were seen repeatedly and the correlations are perfect. The antheridial tube opens at the culmination of a period of gradual development which has been completely traced. After it has emptied its contents it immediately disappears. The oosphere has likewise passed through a series of remarkable but perfectly graded conditions with all the steps of development clearly shown. Coincident with the opening of the antheridial tube certain marked changes appear in the cytoplasm; the oospore wall is formed, the ooplasm immediately becomes much vacuolate where it was previously dense and uniformly constant in character. The discharge from the antheridial tube introduces into the oosphere a large number of nuclei clearly different in form from those previously there. These sperm nuclei are seen in all positions of exit from the tube, and finally become so distributed as to indicate with certainty that they approach the female nuclei.

At a stage positively older (judging by the development of the primitive wall), the oosphere is found full of fusing pairs of nuclei. That these are not nuclei dividing amitotically is proved by the number of nuclei in the oospore decreasing rather than increasing, and also by the evidence presented through detailed study.

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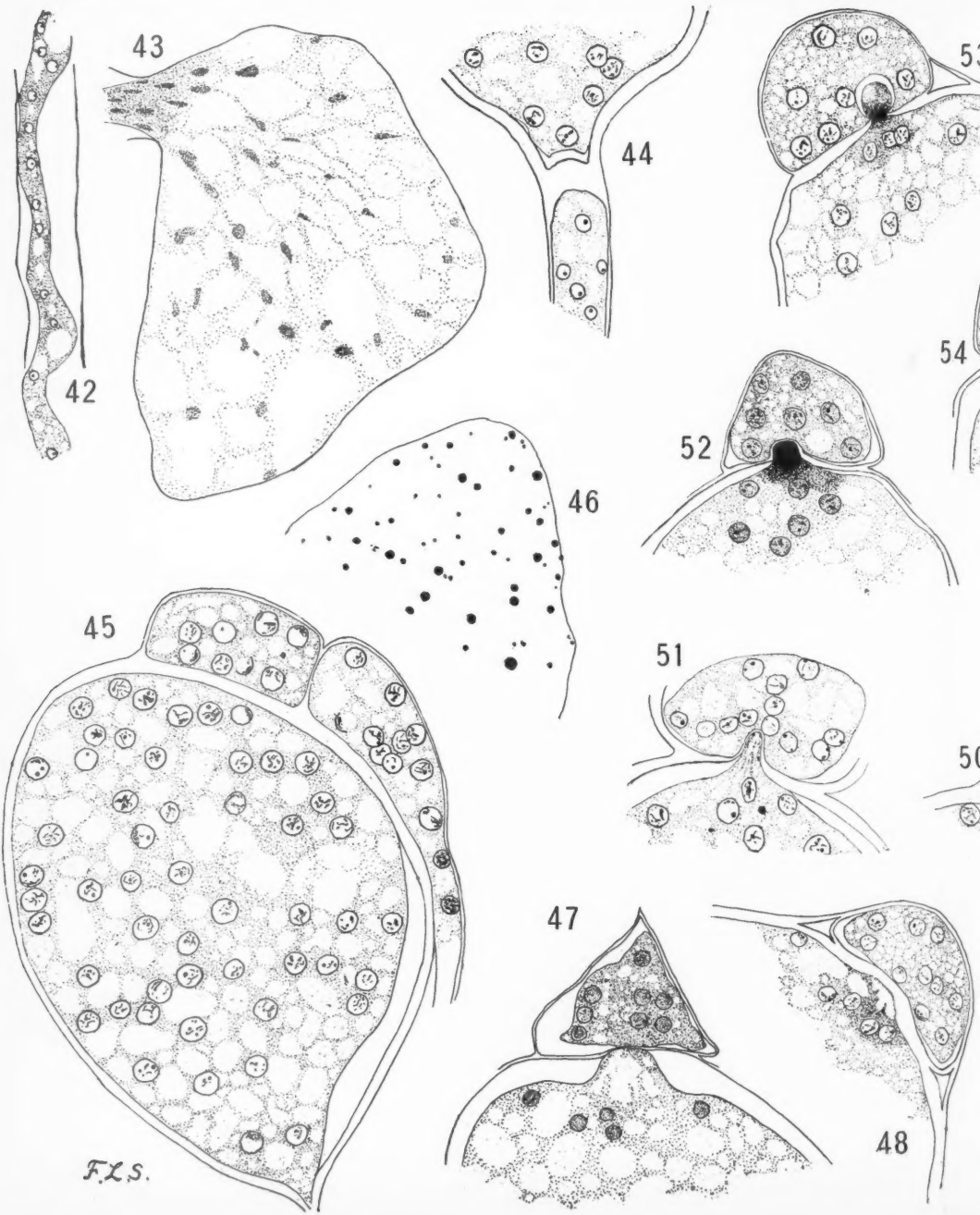
[*To be concluded.*]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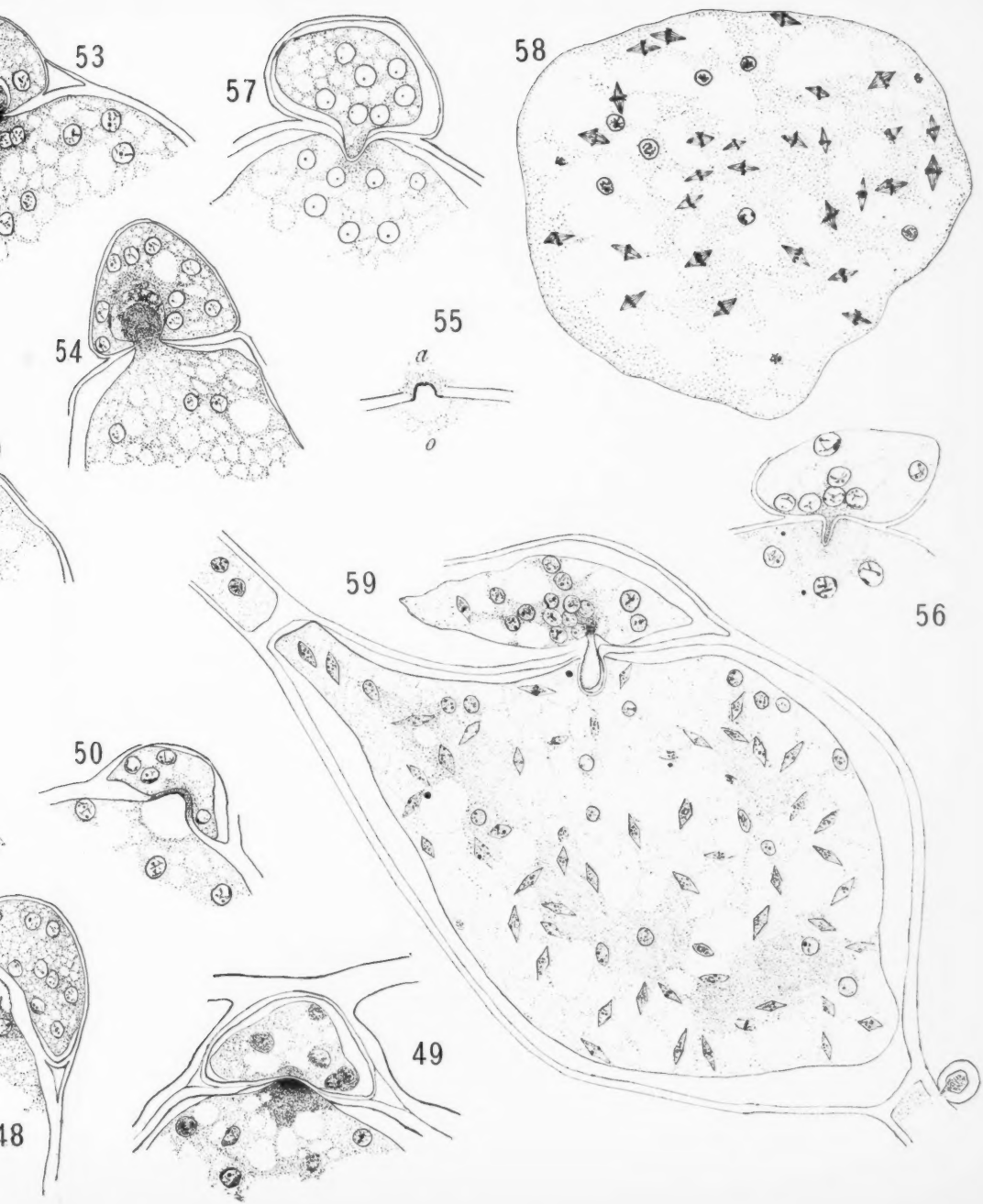

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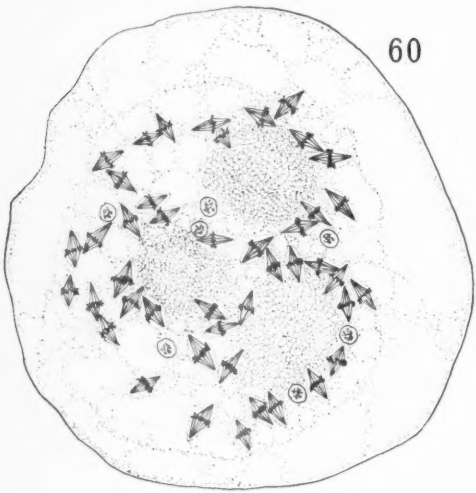

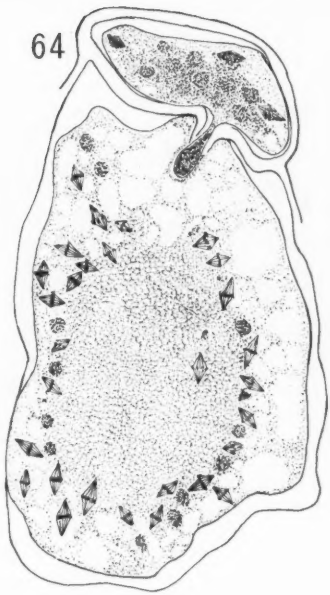

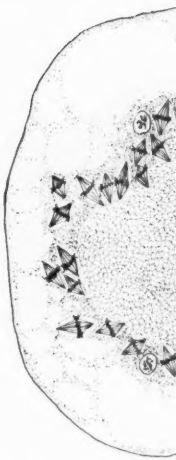

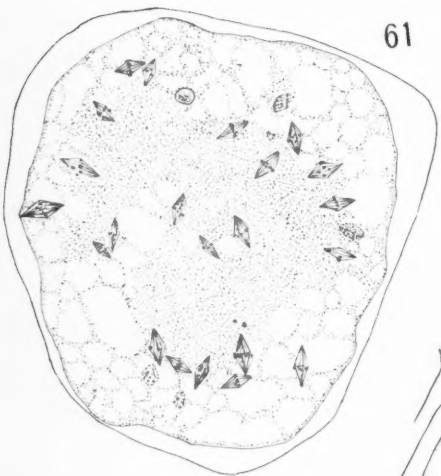

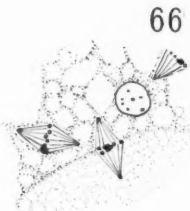

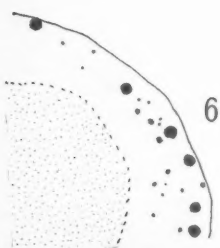

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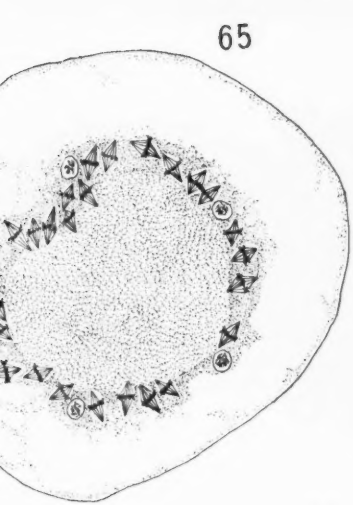

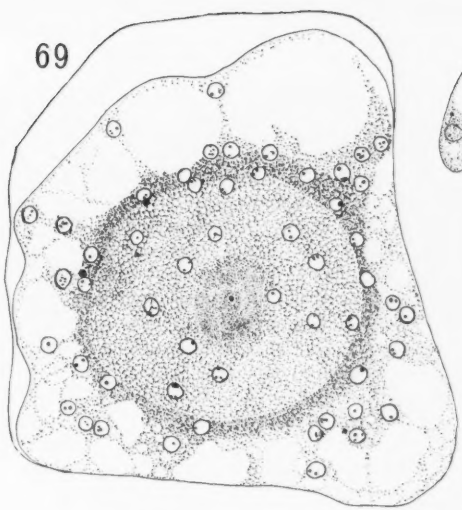

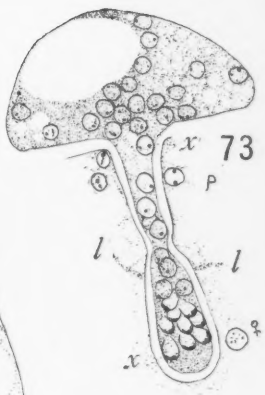

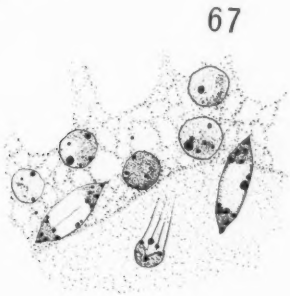

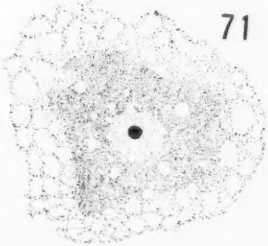

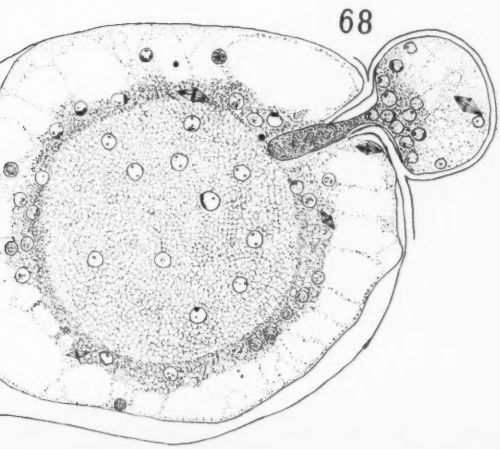

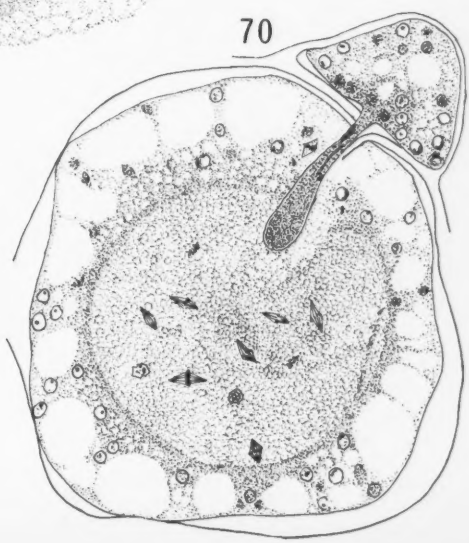

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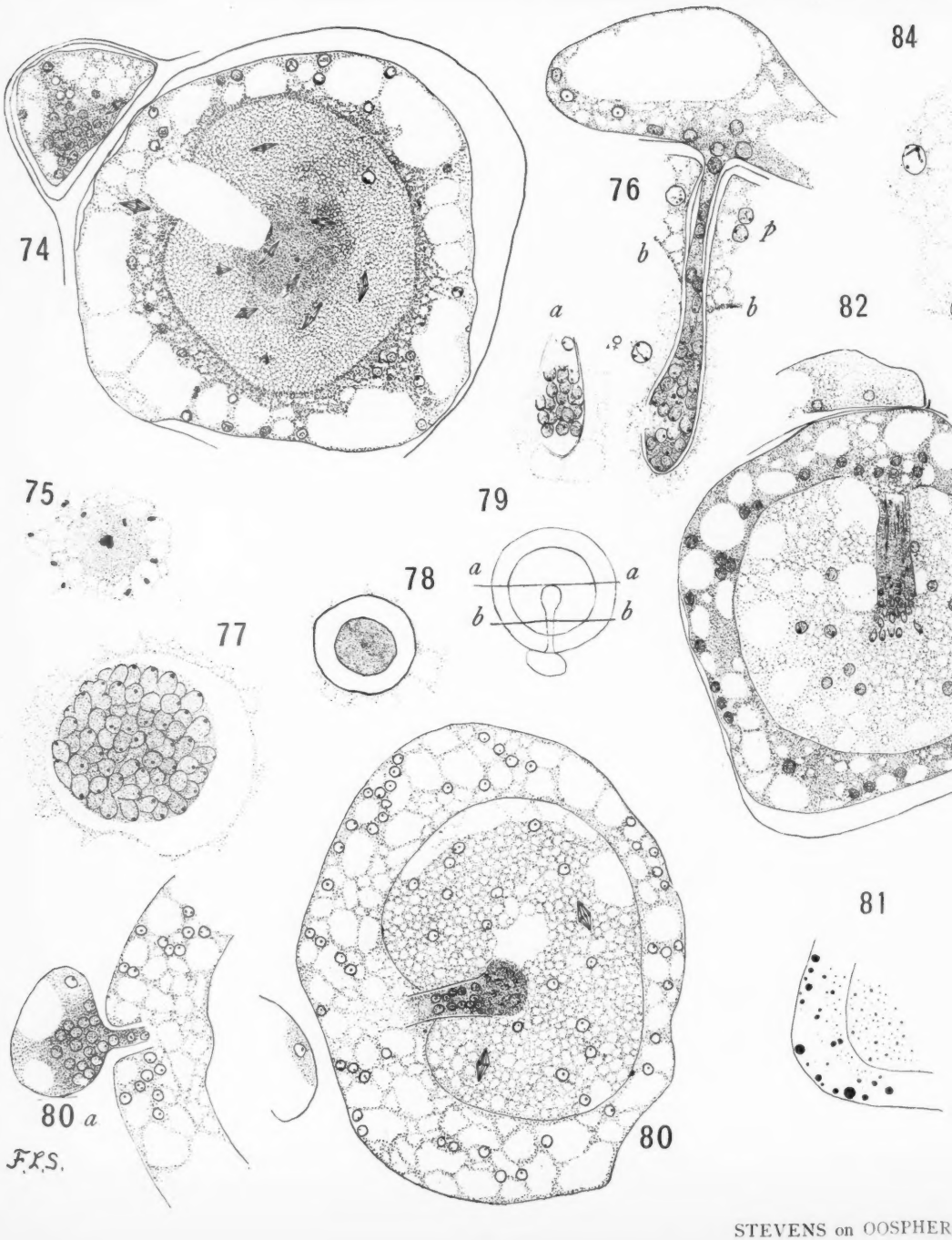
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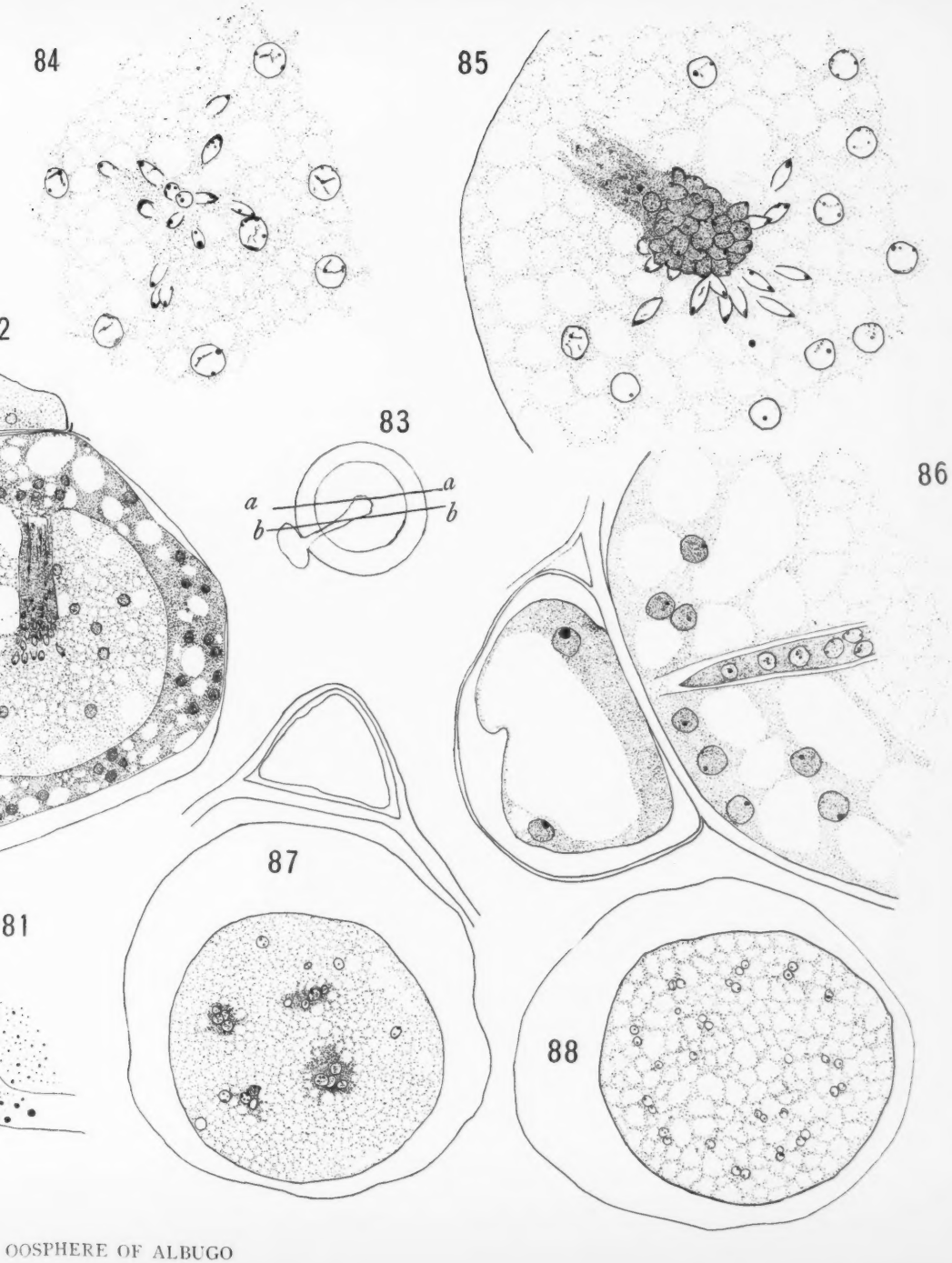

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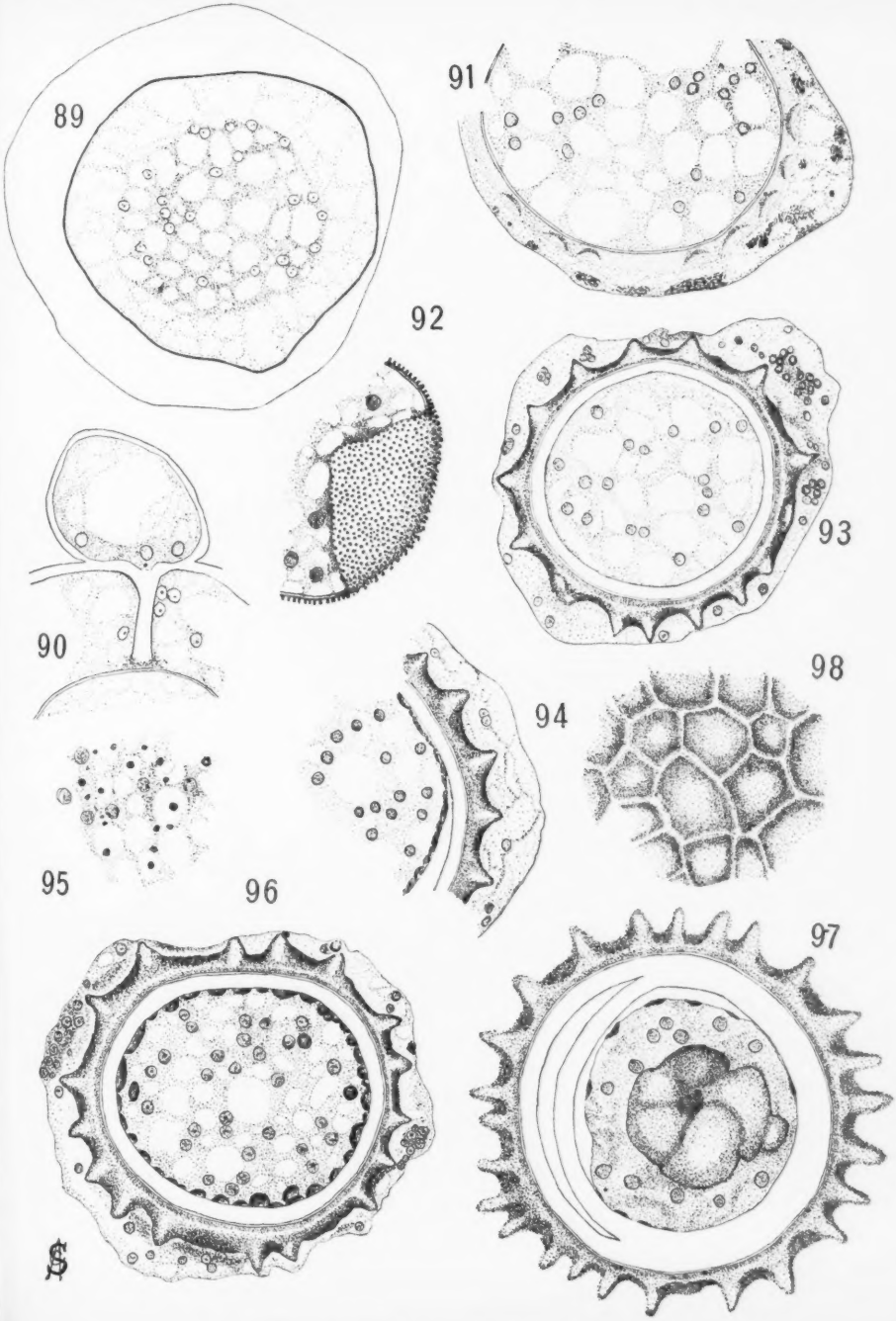

STEVENS on OOSPHERE OF ALBUGO

A BACTERIAL DISEASE OF THE SUGAR BEET.[1]

CLARA A. CUNNINGHAM.

(WITH PLATES XVI–XX)

IN the autumn of 1890 Professor H. A. Huston, chemist of the Indiana Experiment Station, noticed that the analyses of some sugar beets showed a much lower per cent. of sugar than others, and the difference seemed to be associated with a slight change from the usual appearance of the tissues of the root. This observation led to a microscopical examination of the affected beets by Dr. J. C. Arthur, who discovered the presence of bacteria in the tissues, to which, after further study, was attributed their abnormal condition. During the year 1891–2 the characteristics of the disease were studied by Dr. Arthur and Miss Katherine E. Golden, and the results published in the form of a bulletin in 1892.[2]

This preliminary series of investigations determined that the disease was associated with a specific germ, which could be readily isolated from the diseased tissue.

No similar disease of the beet had been reported from any other locality in America at the time of the publication of this work. Dr. Ernst Kramer, in 1891, reported a bacterial disease of the beet root attacking the fodder beets of Russia, and almost simultaneously Dr. Paul Sorauer, of Germany, reported a disease of the sugar beet of that country. In the *Export* of 1894,[3] Dr. Sorauer gives his opinion that the disease of the fodder beet, named by Kramer "bacteriosis gummosis," and that of the sugar beet similarly named by himself "bacteriose gummosis,"

[1] Read before the Society for the Promotion of Agricultural Science at the Boston meeting, August 1898.

[2] Diseases of the sugar-beet root. Purdue University Agric. Exper. Station, Bulletin no. 39.

[3] Export, 1894, no. 30.

are identical, and, perhaps, very closely related to the bacterial disease of sugar cane known as "sereh."

The diseased beets, as observed in Russia, are described as having dried leaves with withered heart leaves. The roots of badly diseased beets were so tough they could scarcely be broken, the broken surfaces soon turning black. These beets produced a pathogenic effect on cattle to which they were fed. Many of the diseased beets, when first sectioned, appeared perfectly sound, but after a few minutes the fibrovascular bundles turned dark and a syrup-like gum exuded from the cells. In other beets the tissue was sometimes completely broken down.

Dr. Sorauer says: "The similarity between the beet and the sugar-cane disease 'sereh' consists in the destruction of the cane sugar and the increase of the invert sugar as well as in the coloring of the vascular bundles and the entrance of bacteria." He also believes that the disease discovered in America by Arthur and Golden may be the same as that determined by Kramer and himself in Europe.

Mr. Walter Busse, in 1895, took up anew the study of the *bacteriosis gummosis* of the sugar beet, the material for study being sent him by Dr. Sorauer. In describing the diseased root he speaks of the gum-like fluid as follows: "Soon after the drops appear on the surface of the sectioned beet they are covered by a thin black membrane, which consists of small, black, round bodies of different sizes."

The aim of Mr. Busse was, first, to determine the form of bacterium common to all the diseased beets by the separation of the germ from the diseased tissue; and, second, to demonstrate that this germ was the specific cause of the disease by inoculating healthy beets with the germ. In the first series of experiments three germs were isolated. Two of these were discarded and the third form was kept for further observation. This form appeared as short rods 1.72–2 μ long and 0.8 μ–0.9 μ broad. They were actively motile, and grew well in cane or grape sugar solutions, producing an abundance of gas. This form was lost, but from other diseased beets a second gas-producing form was

isolated, resembling the first in form and arrangement, but smaller, being 1.5 μ–1.75 μ long and 0.7–0.8 μ broad. This form produced an acid reaction on different media, did not liquefy gelatin, and grew better at 12°–14° than at 22°. Mr. Busse is inclined to believe that the second form is a variety of the first, which he designates as *Bacillus Betæ*. He has demonstrated that this second form produces the disease known as *bacteriosis gummosis*, and believes that this germ is a saprophyte which becomes a parasite in the tissues of the beet.

Erwin F. Smith,[4] in speaking of the bacterial diseases of the sugar beet as reported from Europe and America, is of the opinion that the diseased condition of the beets studied by Arthur and Golden is due to some other cause than a bacterial one. He states that it is highly improbable that the root could be attacked by an organism which invades its tissues and yet does not break them down. Mention is made of the fact of the presence of small bodies in the tissue of healthy beet roots which have the appearence of bacteria, but which are probably crystalloid bodies. A paper by Dr. Smith was presented at the meeting of the Society for Plant Morphology and Physiology in December 1897, calling attention to the "existence, in parts of the United States, of a disease of the sugar beet resembling if not identical with that described by Kramer and Sorauer in 1891–2, and more recently by Busse."

In the fall of 1896 I had the opportunity to continue the investigation of the bacterial disease of the sugar beet observed in Indiana in 1890–1. Much of the value of my experimental study of this disease is due to the suggestions of Dr. Arthur, to whom I am indebted for kindly help and criticism of my work. I also desire to express my gratitude to Professor Burrage, Professor Huston, Miss Golden, and Mr. H. L. Bryan, also of Purdue University, for important suggestions. My investigations, which have been continued from 1896 to the present time, have resulted in no positive evidence that the sugar-beet disease of Indiana is the same as that described by Sorauer and Busse

[4] SMITH E. F.: Am. Nat. **30**:716–729. Sept. 1896.

of Europe. The points of similarity will be noted in the following description of the disease and of the germ by which it is produced.

GENERAL DESCRIPTION OF THE DISEASE.

About the middle of September 1896 several diseased beets were found in the field of cultivated beets on the grounds of the Purdue Experiment Station. The disease attacks the whole beet plant, causing a peculiar appearance of the leaves, so that with a little practice the diseased beets can be distinguished readily from the healthy ones as they grow in the field. The outer, older leaves soon die away, and the intermediate and heart leaves are left wrinkled, curled, rather flabby than turgescent, and of a yellowish-green color. This wrinkled appearance is caused by blister-like patches being formed between the veins of the leaf, and the whole has been described as resembling a Savoy cabbage leaf. See photographs of leaves, *plate XVI*, and also photographs of diseased beest, *plates XVII*, and *XVIII, A*.

The appearance of the exterior of a beet root when diseased is not materially different from that of the healthy beet. It is perhaps not quite as brittle. A decisive test for the disease is found in the appearance that the root shows when sectioned. The fibrovascular bundles appear as dark rings in the white flesh. They grow almost black after being exposed to the air for a few minutes. These rings are quite distinct from the cream colored fibrovascular bundles of healthy beets (*plate XIX*).

In 1896 in a field of beets covering an area of about one acre and containing approximately 130,000 beets, eleven badly diseased and several slightly affected ones were found. This was a smaller number than had been found on the same ground in previous years, and can perhaps be accounted for by the climatic conditions being so favorable to plant growth the preceding summer, there being an abundance of rain. The number of diseased beets increased, however, toward harvest time.

Frost seems to be much more injurious to the diseased than

to the healthy beets. The heart leaves of the diseased beets were more easily injured by the frost. It is characteristic of the disease that the leaves of badly diseased roots die away until no green leaves remain, leaving an apparently dead root in the soil, though its tissues will be found to be firm and not in the least broken down. The early frosts hasten the destruction of the leaves. Both diseased and healthy roots show an acid reaction, the diseased seeming slightly more acid than the healthy.

ETIOLOGICAL HISTOLOGY.

Comparative study was made of sections taken from both diseased beet roots and healthy beet roots, also sections from leaf and leaf-stalk of both diseased and healthy beets. In all these sections small round bodies were seen in the cell substance. These bodies were found to be protein by their reaction to iodine. They measured from 2–4 μ in diameter and turned yellow when treated with iodine. In the tissues of diseased beets other bodies were found which were smaller and of a different refractive power and arrangement. These bodies stained in gentian violet like bacteria, and looked almost like micrococci when imbedded in the cell substance, but when free in the water were easily distinguished as small motile bacilli.

SEPARATION OF THE SPECIFIC GERM.

The first steps in the separation of the germ were as follows. A diseased beet was selected, a thin knife sterilized in the flame and used to remove all parts of the beet exposed to the air. A small piece of beet was then removed from the heart of the root and transferred by means of a sterilized platinum wire to tubes of melted gelatin or agar. The first series of cultures was made by inoculating gelatin tubes with pieces of diseased tissue, at the same time inoculating a number of tubes in the same manner with pieces of healthy beet as a control. The following tables give the results of a series of such inoculations. The first shows the results of cultures made from diseased tissue; the second the results of cultures made from healthy tissue.

TABLE I.

CULTURES ON ARTIFICIAL MEDIA FROM DISEASED AND HEALTHY BEETS.

Dates and media	Number of cultures	Results	
FROM DISEASED BEETS.			
Sept. 20 Gelatin - -	10	Oct. 5 Characteristic growth	Oct. 10 Contaminated
Oct. 2 Gelatin - -	3	Oct. 4 Characteristic growth	Dec. 10 Still pure
Oct. 24 Glycerin gelatin	4	Oct. 28 Characteristic growth	
Oct. 24 Glucose gelatin	4	Oct. 28 Characteristic growth	
FROM HEALTHY BEETS.			
Sept. 20 Gelatin - -	4	Oct. 5 No growth	Oct. 10 No growth
Oct. 2 Gelatin - -	3	Oct. 4[5] No growth	Dec. 10 No growth
Oct. 24 Glycerin gelatin	4	Oct. 28 No growth	
Oct. 24 Glucose gelatin	0		

All the above cultures were made in standard gelatin not titrated or in standard agar to which had been added 5 per cent. glycerin or 5 per cent. glucose. The growth in successful inoculations was the same in all cases. Some creamy-white globules grew out on either side of the diseased tissue, and in the course of a few days were surrounded by a lens-shaped capsule or break in the gelatin caused by the gas that was given off in the growth. When the bubbles reached the surface the growth was distributed in white rings around the tube (*plate XX, B''*).

October 15 several cultures were made in standard gelatin. In some of these a growth resulted but no successful transfers were made. One of these cultures was used to inoculate a healthy beet in the field.

[5] Photograph of tubes at this stage shown in *plate XX*.

November 3 stab cultures were made from a tube of glycerin agar inoculated with diseased tissue. These were all contaminated with the exception of one which formed a perfectly colorless layer, gelatinous in consistence, on agar and sterilized beet. This form will be spoken of later.

No growth resulted from a series of cultures made in 10 per cent. cane sugar gelatin.

In December a diseased beet, which was frozen the previous night, was brought in from the field. Pieces of this tissue were transferred with the usual precautions to tubes of melted gelatin to which had been added 5 per cent. of cane sugar. The growth in these cultures was rapid and gas was given off in large quantities. Stab cultures were made from these and appeared exactly uniform, and just the same in appearance as stab agar cultures made directly from unfrozen diseased beet, with one exception in each case. From one of these exceptional tubes the perfectly colorless gelatinous form spoken of above was found. This form was also obtained in one of the stabs taken directly from the diseased beet.

In the above inoculations the growth was much more rapid than in previous cultures, probably because the tissues were broken down by freezing so that the germ could escape more easily into the surrounding medium.

Another series of cultures was made at the same time from a frozen beet in which the disease was produced by inoculation. The growth was similar to that of the preceding series. Transfers taken from these were uniform and similar to those described above.

In these inoculations a plug was removed from the healthy beet root with a sterilized knife, the inoculating material inserted, the plug replaced and covered with cotton. The table on the next page shows the results of inoculations of three beets in the field.

The beet inoculated with diseased tissue was slower in showing the disease than the one inoculated with the germ growing on gelatin, probably because of the time required for the germ to make its way through the cell walls of the tissue.

TABLE II.

RESULTS OF INOCULATING BEETS IN THE FIELD.

Beet	Date of inoculation	Source of germ	October 22	November 19	November 28
1	Oct. 17	Isolated germ	Yellowish color of leaves noticed	Disease quite evident	Removed from the field
2	Oct. 17	Diseased tissue	Slight indication of the disease	Disease quite evident	Removed from the field
3	Oct. 17	Healthy tissue	No change	No change	No change

The heart leaves of the inoculated beets showed the effect of the disease in the slight blister-like areas on their surfaces. The beet inoculated with the gelatin containing the germ, and the one inoculated with healthy tissue, were brought into the laboratory November 28, as the progress of the disease could not be followed out of doors because of injury by frost. The beets were placed in culture jars of water in the greenhouse, where the healthy beet after some time decayed, and the diseased beet developed new leaves which were more or less crinkled and faded, but gradually assumed a smoother and darker green appearance, but the plant was still stunted in growth.

INOCULATIONS IN THE GREENHOUSE.

The method of inoculation of beets in the greenhouse was similar to that of inoculation of beets in the field. As a result of these trials several beets seemed to show the effects of the disease to a slight degree.

DESCRIPTION OF THE GERM.

The germ as isolated from the diseased tissue is a small bacillus measuring from 0.9–1.0–1.3 μ in length, and 0.5–0.8 μ broad. When taken from the culture media the germs are arranged singly or in pairs, and possess individual motion. The germ

seems to revolve more or less irregularly on its axis. The germ stained well with all the common bacterial stains. No process of staining showed the presence of spores or flagella (*plate XX, B*).

EFFECT OF LIGHT ON GROWTH.

The germ grew better in the dark than in the light. Germs taken from old, dried out cultures were smaller than when grown on a moist substratum. Desiccation also injures the capability of the germ for motion. Germs taken from an old culture and examined in a drop of water were less motile than those taken from a fresh bouillon culture.

The germ grows better at a temperature of 12°–14° than at 21°. Stab cultures in agar grew slowly at body temperature. The germ grown in bouillon and exposed to a temperature of 100° for five minutes was killed.

GROWTH OF THE GERM ON DIFFERENT CULTURE MEDIA.

Stab cultures of gelatin showed a thin grayish-white layer on the surface, and extending down the line of inoculation. As the cultures grew older the color darkened to a deep cream. The gelatin was liquefied in the course of several weeks. When melted gelatin was inoculated and then allowed to solidify there was a growth throughout the gelatin in streaks and films. Colonies on gelatin plates were not distinctly outlined and were sometimes accompanied by a disagreeable odor. The germ seemed to grow better on agar than gelatin. Agar to which had been added 3 per cent. of cane sugar or glucose seemed to specially favor its growth. The growth on slant agar was drab-white in color, smooth margins, and a slow and not luxuriant growth. The growth was not viscous.

In agar plates the colonies have their origin in the deeper layers of the agar where they are generally elliptical. When they reach the surface they spread out in their round grayish-white colonies with compact creamy-white centers. In bouillon growth is observed after two or three days. No turbidity of the fluid was observed, but a sediment was deposited in the bottom

of the tube. Masses of zoogloœa were sometimes found in old bouillon cultures. The germ grew well on sterilized sugar beet, and deposited a sediment in sterilized beet juice. The germ grew on sterilized apple, potato, and turnip. A raw potato was broken open and inoculated with the germ. There was a slight growth developed. A raw sugar beet was inoculated. The germ grew, causing a black coloration of the fibrovascular bundles, and in a microscopical examination was seen to have entered the tissue.

NITRATE SOLUTION.

This solution was prepared using 1000^{cc} distilled water, 1 gram peptone, and 1 gram potassium nitrate. Tubes of this solution in which the germ had grown for three days when tested showed that the nitrate had been fairly well reduced.

ACID AND ALKALINE MEDIA.

It has been stated that the beet root is acid to the extent of little over 1 per cent. Because of this fact experiments were made with acid and alkaline media in order to determine which of the two would be more favorable to the growth of the germ. In bouillon, to which had been added 1 per cent. malic acid, the solution was made neutral; 5 per cent. acid solutions were not rendered neutral. The solution was not made turbid by the growth of the germ. In 1 per cent. alkaline solution of bouillon the germ was more motile than in 1 per cent. acid solution. In 3 per cent. alkaline solutions the germ was more motile and larger than when grown in acid media, measuring from 1.1–1.9 μ long, and 0.9–1 μ broad. In 5 per cent. alkaline media the sediment deposited was quite viscous. Zoogloœa masses were found more or less abundantly in all the alkaline cultures. These solutions were not made acid in reaction by the growth of the germ.

STARCH SOLUTIONS.

In a solution composed of one part each of starch filtrate and bouillon, the germ grew but the starch was not reduced. These cultures were tested for starch and gave a decided

reaction; they were also tested with Fehling's solution for glucose, but gave no reaction. The germ grew well in wort gelatin.[6] When this gelatin was melted, and after being inoculated was allowed to solidify, the germ grew under the surface, producing bubbles of gas all through the gelatin.

Tests made of bouillon containing cane sugar in which the germ had grown gave a reaction for glucose. In order to determine if the enzyme existed outside the cell, a solution in which the germ had grown was filtered through a porous cup. This filtrate added to a 5 per cent. cane sugar solution, and tested with Fehling's solution for glucose gave no reaction. Further experiments are necessary before deciding definitely in regard to the enzyme properties of the germ.

CELLULOSE SOLUTIONS.

As the germ penetrates the cell wall of the plant in some way, experiments were made to determine its effect on cellulose. For this a special nutrient solution was prepared as follows:[7]

Distilled water,	250 cc.
Pepsin,	2.5 gram.
Magnesium sulfate,	.45 "
Calcium phosphate,	.45 "
Ammonium sulfate,	2.05 "
Sodium chlorid,	1.25 "
Beef extract,	1.25 "
Swedish filter.	

In this solution the growth was very slow, and a very small amount of gas was produced, and the cellulose slightly broken down.

FERMENTATION.[8]

Because of the abundance of gas produced by the germ in its growth, special fermentation solutions were prepared. The

[6] Wort gelatin was made by adding 10 per cent. of gelatin to wort.

[7] Sur la fermentation de la cellulose. Centralblatt für Parasitenkunde **2**: 358. 1896.

[8] PAMMEL, L. H. and EMMA: A report concerning gases produced by bacteria in fermentation. Centralb. f. Parasitenkunde **2**: 707. Dec. 1896.

gas produced by the growth of the germ in these solutions was analyzed by Mr. H. S. Bryan, of Purdue University, the results of which are given in Table III. The tests were made for CO_2, O, N, NH_4, and CO, the difference being considered as hydrogen. In the first analysis a much larger amount of nitrogen was obtained than in any of the other cultures. The cultures for this analysis were several weeks old, and were perhaps not trustworthy.

There were some irregularities in the amount of gas produced, which cannot be accounted for. At one time 2 per cent. cane sugar bouillon containing no pepton when inoculated gave a large amount of gas, 20^{cc} being collected in each fermentation tube; 2 per cent. cane sugar bouillon containing no pepton, inoculated at another time under exactly similar conditions, gave only 2^{cc} of gas in each fermentation tube.

The germ grown in bouillon to which had been added 2 per cent. glucose at one time gave a very large amount of gas; at another time there was not enough gas produced to be analyzed. The gas produced by the germ as determined by analyses is composed of a very small amount of oxygen, less than 4 per cent., carbon dioxide approximately 44 per cent., nitrogen 17 per cent., and hydrogen approximately 30 per cent. Fermentation was produced in sterilized beet juice, Pasteur's solution, and maple sap. No fermentation was produced by the growth of the germ in bouillon to which no sugar had been added.

SUMMARY.

It has been determined that a microscopical examination of the tissues of diseased beets reveals the presence of bacteria in the cells of the plant. The tissues of the plant are not broken down, and the bacteria in all parts of the plant appear to be the same. Transfers of diseased tissue to the healthy beet root resulted in changed appearances of the plant which indicated almost certainly that the disease was transmitted.

The manner in which the germ finds entrance to the plant has not been determined. The conditions most favorable to the

TABLE III.

ANALYSIS OF THE GAS PRODUCED BY THE SUGAR BEET GERM.

Date of Inoculation	No. of tubes	Solution	Amount of gas	Results of analysis				
				CO_2	O	H	N	NH_4 and CO
Dec. 18	3	5 % cane bouillon	Dec. 21. 4cc	25%	a trace	a trace	50–60%	none
Jan. 18	several	2 % cane bouillon	Jan. 22. 20	67	.5%	20.6%	11.9	"
Jan. 25	3	2 % cane bouillon	Jan. 29. 15	61.8	.69	27.5	10.1	"
Jan. 25	3	2 % glucose bouillon	Jan. 29. 20	38.7	3.6	36.5	21.2	"
Jan. 25	2	2 % lactose bouillon	Not enough gas to be analyzed					
Jan. 25	3	2 % cane bouillon, no pepton	Feb. 2. 20	37.63	7.22	21.64		"
Feb. 3	7	2 % glucose bouillon	Feb. 11. 2	18	1.7	explosion probably H	33.51	"
Feb. 3	3	2 % cane bouillon, no pepton	Feb. 11. 2	No analysis made				
March 29	several	5 % cane bouillon, with 3 % calcium chlorid	April 26 10	44.23	1.92	33.65	14.02	"
March 29		7 % acid wort solution	April 26 15	53.27	.9	31.77	14.09	"
April 1	several	Cellulose solution	Small	No analysis made				

attack are those resulting from drought with succeeding low temperature.

The fact that the germ breaks down cellulose slowly explains the manner of its progress from one cell to another.

Experiments have shown that the germ in a medium of low per cent. acid grows nearly or quite as well as in one of alkaline nature, so that the acid element of the beet root does not offer material resistance to the germ.

The germ converts cane sugar to glucose in the process of producing gas. The amount of gas produced is not constant, but the reasons for this irregularity have not been determined.

The germ grows well with any form of sugar and especially well in media containing cane sugar. This fact makes it seem probable that the germ is especially at home on those media which contain sugar in some form, although it will keep alive on media without sugar, and after cultivation for a time on such media will adapt itself to the conditions presented.

ANOTHER ORGANISM SEPARATED FROM THE SUGAR BEET.

The colorless gelatinous form separated from the beet root in connection with the disease germ was at first thought to be an undescribed germ or rather the product of a germ, for only a few bacterial bodies could be detected under the microscope even when comparatively large masses of the substance were placed in the field. The organism appeared as small bacilli or micrococci.

The mass resembles the form of Leuconostoc so common in the vicinity of sugar refineries. Under the microscope, however, no streptococci were found, which characterizes Leuconostoc under the microscope. The gelatinous substance is soluble in water and alcohol; in the latter it turns to a milk-white substance before it dissolves. The substance increased rapidly in bulk when grown on sterilized beet. The mass did not dry out for months after the substratum had become dry and hard.

The substance grew well on 10 per cent. cane sugar agar. The growth was slow at first, but after a week or two masses

measuring a quarter of an inch in thickness and three fourths of an inch in circumference, collected on the surface of the medium in stab and slant cultures. In case of stab cultures the agar was broken vertically along the line of inoculation. The colorless growth followed this break in the agar, and as the substratum became hard the mass collected as a colorless semi-fluid in the bottom of the test tube.

On slant agar there was a thin colorless layer, imparting a fluorescent hue to the medium. In agar plate cultures the organism formed small round colonies about the size of a pin head, resembling a small drop of water. These colonies were sometimes found with the disease germ, in plate cultures taken directly from the beet. It also grew on sterilized potato, and to some extent on gelatin. Immediately after separation from the beet root the organism produced fermentation, but the power was lost after a time. Staining revealed only a structureless mass containing a few bacteria-like bodies.

Desiccation has little effect on the substance. Sections of beet on which the organism was growing have been kept in the laboratory until they are quite dried out, and the gelatinous mass is still apparent.

If this is indeed a form of Leuconostoc, it is interesting to find it in diseased beet roots.

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EXPLANATION OF PLATES XVI–XX.

PLATE XVI.

Leaves of healthy and diseased beets, as they appeared when brought in from the field. The three diseased leaves can readily be distinguished from the two healthy ones because of their blistered and crinkled surface.

PLATE XVII.

A diseased beet brought in from the field. The root is quite firm, none of the tissue being broken down. The leaves hanging down are quite dead and dry. The erect heart leaves are alive and show the characteristic crinkled surfaces.

PLATE XVIII.

A, a diseased beet with the dead leaves removed. *B*, a healthy beet of same size and stage of growth, also with the dead leaves removed.

PLATE XIX.

A, cross sections through the crown of healthy and diseased beet roots. *a*, healthy root. *b*, diseased root, characterized by the black rings of vascular tissue.

B, cross sections through the central portion of the same diseased and healthy beet roots. *a*, healthy root. *b*, diseased root.

C, cross sections near the tip of the same roots. *a*, healthy root. *b*, diseased root.

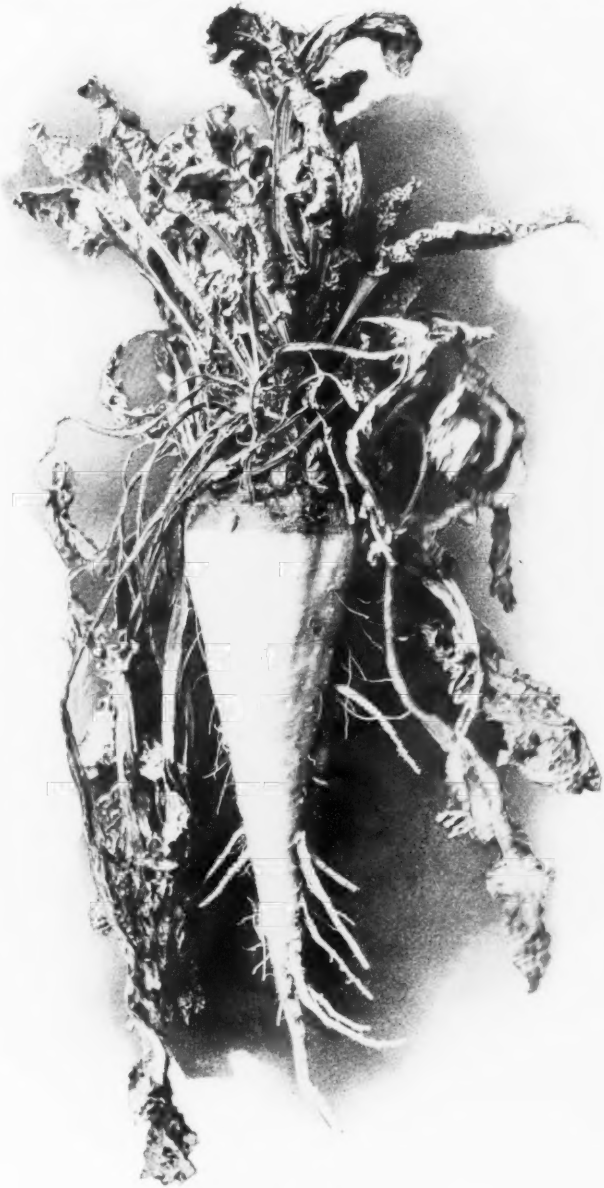
PLATE XX.

A, longitudinal sections of the same healthy and diseased beet roots figured in the preceding plate. *a*, healthy beet. *b*, diseased root.

A'', tube of gelatin inoculated with a piece of healthy beet. *B''*, similar tube inoculated with a piece of diseased tissue. The photographs were taken after being inoculated two weeks. In *A* no growth appeared. In *B* the small globules of growth can be seen, breaking the agar and pushing it upward by the production of gas.

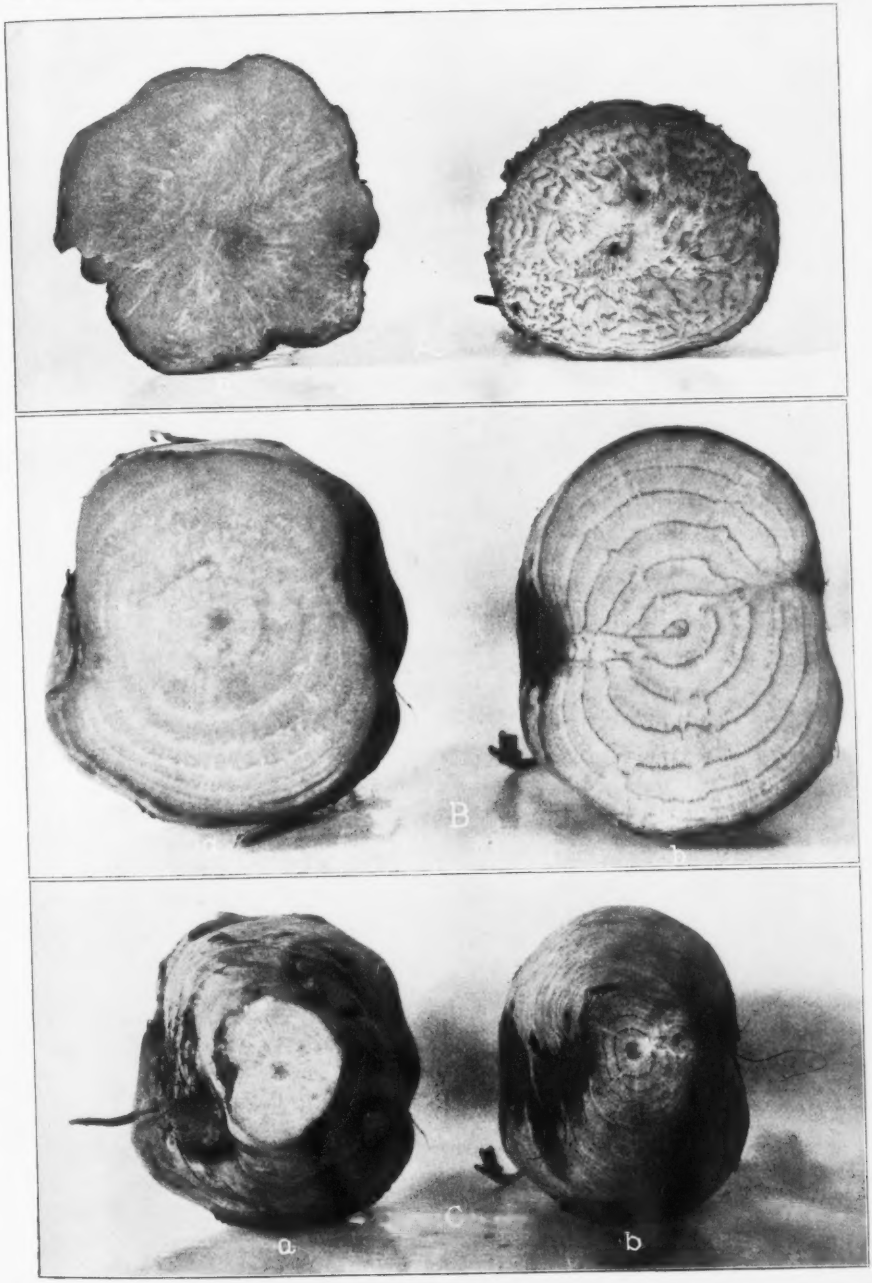
B, the disease germ stained with carbol fuchsin; imperfectly photographed.

CUNNINGHAM on SUGAR BEET



CUNNINGHAM on SUGAR BEET

CUNNINGHAM on SUGAR BEET



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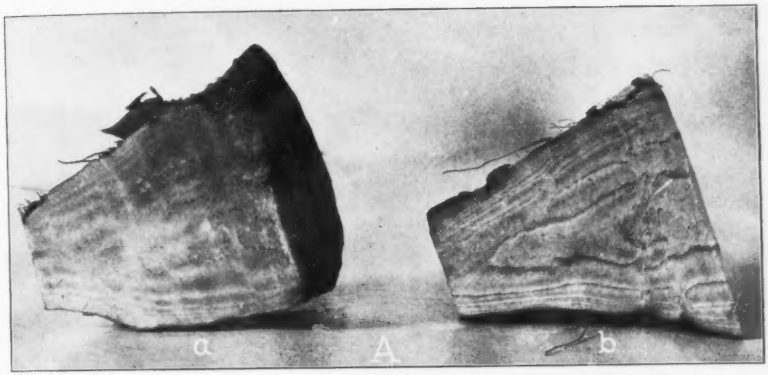
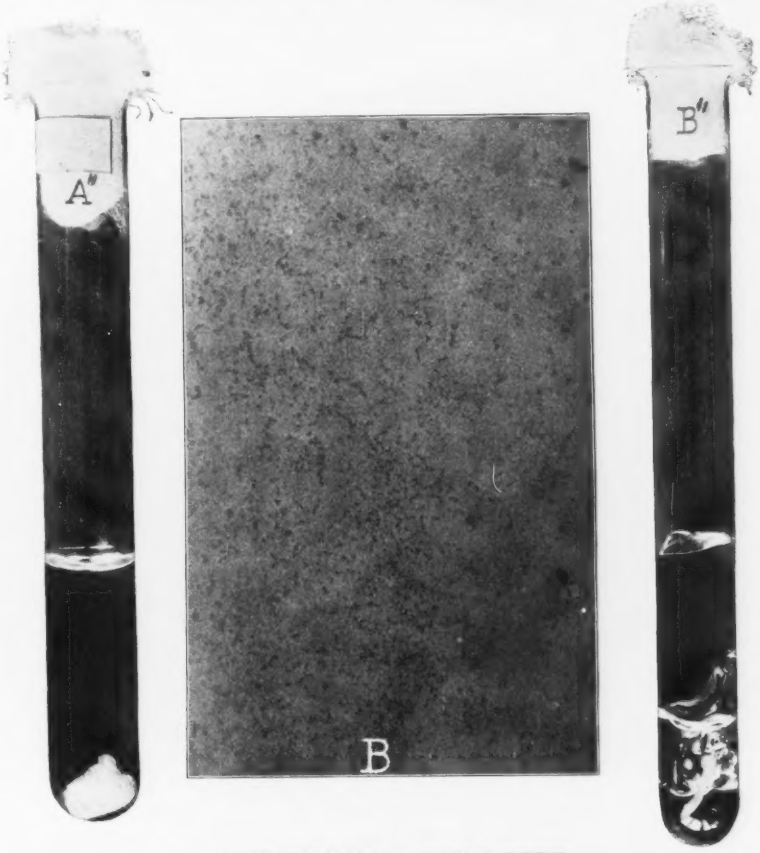




CUNNINGHAM on SUGAR BEET

REVISION OF THE NORTH AMERICAN SPECIES OF TEPHROSIA.

B. L. ROBINSON.

WHILE the greater part of the difficulties of the genus Tephrosia lie, happily, beyond the geographical limits of the North American flora, yet the dozen species which inhabit sandy regions in our southern states are sufficiently variable in their foliage and similar in their floral and carpological structure to lead to diverse views on their specific limits and proper arrangement. Miss Vail's recent monograph[1] of these species, although abounding in long and detailed descriptions, fails to bring out clearly the primary divisions of the genus. The introductory synopsis — which, although arranged in perfect accord with the natural sequence of the species in the paper, is styled an "artificial key" — is based chiefly upon the length and density of the inflorescence, features which are too variable and confluent to furnish diagnostic characters of the first rank. It seems to the writer that the sympodial and monopodial structure of the stem offers a difference much more evident and, indeed, sufficiently fundamental to divide our species at once into two well-marked sections. After this first division the size of the flowers, density of the inflorescence, and nature of the foliage may be employed with greater diagnostic effect.

In the limitation of species it is believed that Miss Vail's *Cracca floridana* is only separated artificially from her *C. Smallii*. The distinctions adduced are chiefly the "truly prostrate" habit of the former species, and its narrower, more numerous leaflets. As to the breadth of the leaflets, this is (according to Miss Vail) 6 to 14^{mm} in *C. Smallii*, and 5 to 12^{mm} in *C. floridana*, surely no very striking difference. In number the leaflets are said to vary in *C. Smallii* from 3 to 11, while in Mr. Nash's

[1] A revision of the North American species of the genus Cracca. Bull. Torr. Bot. Club **22**: 25–36.

authenticated specimens of *C. floridana* they vary from 7 to 13, and had these specimens been younger, it is safe to surmise, leaves with fewer leaflets would have been found, an inference amply supported by the development in related species. Regarding the supposed assurgent habit of *C. Smallii* (a species not only distinguished in the field, but called to scientific notice by Mr. A. H. Curtiss), it may be said that the main stems bear evidence of being prostrate except near the tip. This is shown not only by the curvature and position of the leaves and peduncles, but by the almost constant presence of clinging particles of sand which adhere, even in the type specimens, to the lower surface of the stem, and are lacking upon the upper surface. This position of the stem is fully confirmed by field notes kindly furnished by Mr. Curtiss. The assurgent character is, therefore, confined to the leaves (which are said to be somewhat ascending but less erect than in *T. ambigua*), the peduncles, and the growing tips of the stems. On the other hand, specimens of *C. floridana*, although said to have prostrate stems and leaves, give by no means the impression of a closely prostrate plant, an idea which is at once conveyed by specimens of *T. chrysophylla*. In the absence of more telling morphological features the two species *C. floridana* and *C. Smallii* are here united.

Miss Vail is certainly in error in interpreting Shuttleworth's *T. angustissima*, her characterization evidently being drawn from Mr. Curtiss' plants so named. Shuttleworth's type, collected on the Miami river by Rugel, is in the Gray herbarium, and is clearly a linear-leaved form of *T. purpurea* Pers., with which it shares the characteristic small flowers, of which one or two are axillary, and the rest borne in a very slender raceme. On the other hand Curtiss' nos. 584 and 5708, just mentioned, appear to be nothing but smoothish narrow-leaved *T. ambigua*, not differing by a single morphological character of specific value. In her key (p. 26), Miss Vail distinguishes this plant (her *Cracca angustissima*) from *T. ambigua* on the ground that it is *erect*, and has linear leaflets, but in her description (p. 32) it is characterized as *prostrate*.

T. Rugelii Shuttleworth is an interesting and hitherto undescribed species possessing the stem-structure of § BRISSONIA but the habit and foliage of *T. chrysophylla*, which is of § REINERIA.

T. tenella Gray seems to have been founded upon a juvenile state of *T. purpurea*, to which (under the name of *T. leptostachya* DC.) it was reduced by Bentham in Mart. Fl. Bras. **15**[1]: 48. *T. purpurea*, however, is highly variable, at least in foliage, and certainly merits further study with more copious material than is now available.

The writer is grateful to Drs. Britton and Small for the loan of the larger part of the North American Tephrosias from the herbarium of the New York Botanical Garden. These have been of material assistance in the present revision.

As the genus is here interpreted in its generally accepted significance, it is useless to take space for a generic description.

Besides the species here described, the tropical *T. cinerea* Pers. has been found in the southern states, but only on ballast ground (Alabama, *Dr. C. Mohr*). It is of the § REINERIA, and has rather numerous narrowly oblanceolate-linear leaflets, which are hoary-pubescent upon both surfaces.

§ 1. BRISSONIA DC. Stem monopodial: racemes terminal or axillary, never opposite the leaves: flowers (in our species) large, with petals 1 to 1.7 [cm] long.—Prodr. **2**: 249, in part; Taubert in Engl. & Prantl, Pflanzenf. **3**: Ab. 3. 269. *Brissonia* Necker, Elem. **3**: 36.

* Pods glabrous at maturity: racemes loose: southwestern.

T. LEIOCARPA Gray. Undershrub, cinereous with fine appressed pubescence: stems several, 4 to 6 [dm] high, suberect: leaves petiolate, 15–19-foliolate; leaflets narrowly oblong, 1.6 to 3.2 [cm] in length: racemes both terminal and axillary: pods 5 to 5.7 [cm] long, about 10-seeded.—Pl. Wright. **2**: 36; Walp. Ann. **4**: 489. *Cracca leiocarpa* Kuntze, Rev. Gen. **1**: 175; Vail, Bull. Torr. Club **22**: 28 (where type locality is erroneously stated to be New Mexico).—Sonoita valley, close to the southern boundary of

Arizona, *Wright*, no. 965, *Rothrock*, no. 685. (Northern Mexico.)

* * Pods permanently pubescent or puberulent: inflorescence short and dense.

+– Calyx-lobes ovate-lanceolate to lanceolate: pubescence gray: petioles rarely 6^{mm} in length.

T. VIRGINIANA Pers. (GOAT'S RUE.) Stems several, 3 to 4.5^{dm} high, erect from a stout knotted lignescent root: pubescence fine, soft, somewhat variable in quantity, often copious toward the summit: leaflets 11 to 23, oblong to elliptical, green and scarcely pubescent above, somewhat paler and soft villous beneath, 2.5 to 3.2^{cm} long; petioles very short; stipules caducous: flowers borne partly in pairs or singly in the upper axils but chiefly in a short dense raceme little raised above the surrounding leaves: calyx hairy, the teeth caudate-acuminate: petals white or pale yellow with purplish tinge: hirsute pods soon spreading or divaricate.—Syn. **2**: 329; Pursh, Fl. **2**: 489; Ell. Sk. **2**: 245; Torr. & Gray, Fl. **1**: 295; Wats. & Coulter in Gray, Man. ed. 6, 133; Meehan, Nat. Fl. **1**: 81. *pl. 21*. *T. virginica* Bigel. Fl. Bost. ed. 3, 296. *Galega virginica* L. Spec. ed. 2, **2**: 1062; Hill. Veg. Syst. **21**. *pl. 55, f. 1*. *Cracca virginiana* L. Spec. **2**: 752; Vail, l. c. 27.—Dry open woods especially in sandy soil, common; New England to the north shore of Lake Erie, thence to Texas and Florida; fl. May, June; fr. July to September.

Var. HOLOSERICEA Torr. & Gray. Leaflets inclining to be narrow and acute; pubescence more copious, long and silky or even woolly on the pods.—Fl. **1**: 296. *T. holosericea* Nutt. Jour. Acad. Philad. **7**: 105. *Cracca virginiana* var. *holosericea* Vail, l. c.—Arkansas, *Nuttall*, *Marcy Exp.*, and Texas, *Hall*. Similar but less marked forms in Wisconsin and W. New York.

+– +– Calyx-lobes very narrow, almost filiform: pubescence tawny.

++ Petioles 1.7 to 4.2^{cm} long: leaflets oblong: southwestern.

T. LEUCANTHA HBK. Erect, branched from a lignescent base: leaflets 15 to 20, oblong, appressed-villous on both surfaces, nearly concolorous, 1.9 to 3.2^{cm} long: racemes capitate, pedunculate, chiefly terminal; linear filiform bracts considerably exceeding the buds: petals white with or without a purple tinge: pods

narrow, 5 to 6.2 cm long, soon divaricate.—Nov. Gen. & Spec. 6: 460. *pl. 577;* Gray, Pl. Wright. **2:** 36; Torr. Bot. Mex. Bound. 51. *Cracca leucantha* Kuntze, l. c.; Vail, l. c.—Mountainous regions in S. Arizona, *Rothrock, Lemmon, Pringle.* (Mex. where first collected by *Humboldt & Bonpland.*)

++ ++ Petioles 4 to 17 mm long: leaflets obovate: Florida.

T. Rugelii SHUTTLEWORTH in herb. Stems several, decumbent or suberect from a lignescent stock, finely appressed-pubescent with bronze-colored hairs: leaves 3–11-foliolate; stipules persistent, 4 to 6 mm long; leaflets obovate, retuse, mucronulate, finely appressed-pubescent and yellowish-green above, decidedly paler, cinereous and very veiny beneath, 8 to 17 mm long, half as broad: flowers borne chiefly in pairs in the upper axils or forming a subcapitate raceme at the summit of the stem: calyx tawny-villous, 5 mm long, its narrow teeth subequal: petals probably purple: pods somewhat falcate, 3.8 cm long, 5 mm broad, tomentulose.—In pine woods on the Manatee river, S.W. Florida, *Rugel,* no. 156, June 1845. Type in herb. Gray. A characteristic and, according to our present knowledge, thoroughly distinct species with the habit of *T. chrysophylla,* from which it differs in its monopodial stem and axillary flowers, as well as in the presence of pubescence upon the upper surface of the leaves.

§ 2. REINERIA DC. l. c. 251, in part. Stem sympodial, the main axis at one or more nodes terminating in a raceme which by the strong development of an axillary bud at its base becomes apparently lateral: some of the racemes thus appear to arise opposite the leaves. (The sympodial structure is tardily developed in *T. purpurea,* which during its first season sometimes produces only a terminal raceme. This species, however, may be readily distinguished from those of the preceding section by its smaller flowers, which are only 6 to 8.5 mm long.)—*Reineria* Moench, Meth. Suppl. 44.

* Flowers large: petals 1 to 1.7 cm long.

+– Leaflets (with rare exceptions) exceeding the short petioles.

+ + Flowers not numerous, borne singly or in pairs at the nodes of the racemes.

= Stem covered at least below with a short dense bronze-colored tomentum: leaflets thickish, of firm or subcoriaceous texture, glabrous and finely reticulated above.

a. Leaves prostrate, essentially sessile; leaflets seldom more than 7.

T. CHRYSOPHYLLA Pursh. Perennial herb with spreading prostrate freely branched somewhat flexuous or geniculate stems, subsessile, 2–7-foliate leaves, and obovate leaflets (1.3 to 3^{cm} long): few-flowered racemes opposite the leaves; peduncles scarcely ancipital, 5 to 8.8^{cm} long: petals white, changing to red: pubescence on the lower surface of the leaflets dense, sericeous, somewhat canescent but with a slight golden sheen: pods 3.4 to 4.2^{cm} long, 8–10-seeded.—Fl. **2**: 489; Ell. Sk. **2**: 246; Torr. & Gray, Fl. **1**: 297; Chapm. Fl. 95. *T. prostrata* Nutt. Gen. **2**: 120. *Cracca chrysophylla* Kuntze, l. c. 174; Vail, l. c. 34.—Dry pine woods, Georgia, *Boykin, Forbes*, to Florida, where apparently common, "and westward" acc. to Chapman, but probably in reference to *T. Smallii.*

Var. **Chapmanni.** Plant smaller, leaflets 6 to 13^{mm} long, half as broad: pods only 1.9^{cm} long, 5–8-seeded.—*Cracca chrysophylla* var. *Chapmanni* Vail, l. c.—St. Josephs, Florida, *Dr. Chapman.*

b. Leaves, at least in some cases, ascending, petiolate; leaflets mostly 7 to 11.

T. Smallii, n. comb.—Similar to the preceding species in many ways, but stouter, with more numerous and longer (oblong or elliptic rather than obovate) leaflets: peduncles decidedly ancipital above, becoming 5 to 20^{cm} in length.—*Cracca intermedia* Small, Bull. Torr. Club. **21**: 303. *C. Smallii* and *C. floridana* Vail, l. c. 33, 35.—Pine barrens in sand, Georgia, *Boykin*, to Florida, *Curtiss, Nash*, and Louisiana, *Dr. Ingalls.* The previous use of *intermedia* in Tephrosia necessitates the adoption of the second specific name.

= = Stems very slender, sparingly pubescent: leaflets rather small, elliptical, thin.

T. HISPIDULA Pers. l. c. Stems several, branched, spreading and ascending from a thickish somewhat fusiform root, finely

pubescent or glabrate; hairs sometimes spreading but usually appressed: leaflets 11 to 17, elliptic-oblong to linear-lanceolate, thinnish, usually deflexed, appressed-pubescent or glabrous above, slightly paler appressed-villous beneath, usually rounded and mucronulate at the apex: petals at maturity purple, sometimes 1.6^{cm} in length: pods 8–10 seeded, covered with short appressed or more often spreading hairs.—Pursh, Fl. **2**: 489; Ell. Sk. **2**: 245; Torr. & Gray, Fl. **1**: 296, excl. vars.; Chapm. Fl. 95. *T. gracilis* Nutt. Gen. **2**: 119. 1818. *T. elegans* Nutt. Jour. Acad. Philad. **7**: 105. *Galega hispidula* Michx. Fl. **2**: 68. *Cracca hispidula* Kuntze, l. c. 175; Vail, l. c. 33.—Sandy barrens, Virginia and N. Carolina, *Curtis*, to Florida and Louisiana, *Hale*.

= = = Stems rather stout, covered with a copious coarse tawny spreading pubescence; leaflets sparingly villous along the midnerve above or appressed-pubescent over the entire upper surface.

T. VILLOSA Pers. l. c.[1] Stem 3 to 9^{dm} long, sprawling, tawny-hirsute: leaflets 3 to 17, elliptic or obovate, rounded and apiculate at the end, villous beneath, more or less appressed-pubescent also on the upper surface, about 2.5^{cm} long, a third to half as broad; rhachis tawny-pubescent: peduncles long, somewhat ancipital; interrupted few-flowered raceme surpassing the leaves: calyx lobes with long filiform tips: petals pale or more often (at least in age) purple red.—*T. spicata* Torr. & Gray, Fl. **1**: 296; Chapm. Fl. 95; Wats. & Coulter in Gray, Man. ed. 6, 133. *T.*

[1] This species was originally described by Michaux (1803) as *Galega villosa*, with a range "a Carolina ad Floridam." There is absolutely no evidence that Michaux regarded his plant as the equivalent of the Asiatic *G. villosa* L. The name *Tephrosia villosa* is first employed by Persoon in his Synopsis (1807) and is there used exclusively for the American plant. This is shown not only by the identical range but by the quoted character, which is taken bodily from Michaux's work. The usage of De Candolle and many more recent European writers, by which the name *T. villosa* Pers. is applied to an East Indian and African species, to which Persoon's description had no reference whatever, is clearly an unwarrantable transposition. It is true that there was an earlier *Galega villosa* than that of Michaux, but this should not invalidate *Tephrosia villosa* Pers., which is clearly applied to the American plant and antedated by no homonym. The plant of the Old World, although possessing an earlier specific name, was not brought under Tephrosia until later and, it is believed, should in that genus receive another specific designation.

paucifolia Nutt. Gen. **2**: 119. ? *T. hispida* DC. Prodr. **2**: 250. *T. mollissima* Bertol. Bot. Miscel. 9: 10. *pl. 3. f. 2*, and Bot. Zeit. **9**: 902, acc. to Gray. *Galega spicata* Walt. Car. 188. *G. villosa* Michx. Fl. **2**: 67. *G. paucifolia* M. A. Curtis, Jour. Bost. Nat. Hist. Soc. **1**: 122. ? *Crafordia bractiata* Raf. Specch. **1**: 156. *Cracca spicata* Kuntze, l. c.; Vail, l. c. 30.— Dry sandy ground, common, Delaware to Florida, west to Arkansas (acc. to Lesquereux) and Louisiana, *Hale;* fl. May to July.

Var. **flexuosa**. Leaflets linear to lance-linear, acute, the terminal one much elongated.— *T. flexuosa* Chapm. acc. to Torr. & Gray, l. c. 297, in synon. *T. hispidula* var. γ Torr. & Gray, l. c. *Cracca spicata* var. *flexuosa* A. M. Vail, l. c.— Florida, *Chapman;* poorly known and perhaps distinct. A similar but nearly glabrous form has been found in Alabama by *Gates*.

++ ++ Flowers numerous, the middle ones borne in threes and fours: leaflets also numerous, 9 to 27, linear-oblong, 2.5 to 3.8^{cm} in length.

T. ONOBRYCHOIDES Nutt. Rather stout for the genus, erect or nearly so: stem terete, geniculate, producing from near the summit a long-peduncled erect many-flowered raceme: leaflets oblong, silky beneath, obtuse or retuse at the apex, cuneate at the base, 2.5 to 3.8^{cm} long, a fourth as wide: flowers pale changing to red or at length purple: pods secund, finely appressed-pubescent.— Jour. Acad. Philad. **7**: 104; Torr. & Gray, Fl. **1**: 296; Engelm. & Gray, Pl. Lindh. 1: 6, 33; Gray in Hall, Pl. Tex. 7; Chapm. Fl. ed. 2, 615. *T. angustifolia* and *T. multiflora* Featherman, Bot. Rep. Louisiana Univ. **1870**: 73, acc. to Gray, Am. Jour. Sci. III., **2**: 375. *Cracca onobrychoides* Kuntze, l. c.; Vail, l. c. 29.— Dry plains, Arkansas, *Nuttall*, *Harvey*, to Louisiana, *Hale*, and Texas.

+ + Petioles longer than the leaflets (rarely equaled by them in *T. Lindheimeri*).

++ Pods 6 to 8.5^{mm} broad: leaflets suborbicular.

T. LINDHEIMERI Gray. Soft-pubescent perennial herb with long reclining branched stems and 5–13-foliolate leaves: leaflets broadly obovate, rounded at the end, subcuneate at the base, soft-sericeous upon the lower surface; linear striate attenuate stipules

very long: flowers rather numerous in erect pedunculate racemes, purple: pods velvety-tomentose.—Pl. Lindh. 2: 172; Torr. Bot. Mex. Bound. 51. *Cracca Lindheimeri* Kuntze, l.c.; Vail, l. c. 28. Sandy prairies, southwestern Texas, *Lindheimer, Wright, Palmer, Havard, Fuchs.* (Adj. Mex. where first collected by *Berlandier.*)

++ ++ Pods about 4^{mm} broad.

T. AMBIGUA Chapm. Stems prostrate or ascending, from a deep woody root, copiously pubescent to nearly glabrous: leaves erect, 3–13-foliolate, petioles 3.8 to 6.3^{cm} long; leaflets obovate to oblong, obtuse or obtusish, 2.5 to 3.8^{cm} long, 6 to 17^{mm} broad, sparingly appressed-pubescent or glabrate above, veins red and surface paler, appressed-pubescent beneath; stipules 4 to 8.5^{mm} in length: peduncles long, ancipital, remotely 3–6-flowered: calyx very small: petals purple: pods narrow, appressed-pubescent, many-seeded.—Fl. 96; Wood, Bot. & Flor. 95. *T. hispidula* var. *β* Torr. & Gray, Fl. 1: 296. *Galega ambigua* M. A. Curtis, Jour. Bost. Nat. Hist. Soc. 1: 121. *Cracca ambigua* Kuntze, l. c. 174; Vail, l. c. 32.—N. Carolina, *Curtis,* to Florida and Mississippi, *Pollard;* common.

Var. **gracillima**. Very slender: leaflets lance-linear, acute or acutish, seldom over 4^{mm} in breadth.—*Cracca angustissima* Vail, l. c. 32, not *T. angustissima* Rugel.—Dry pine barrens near Eau Gallie, Indian river, Florida, *A. H. Curtiss,* nos. 584, 5708; fl. July.

* * Flowers smaller; petals 6 to 8.5^{mm} long.

T. PURPUREA Pers. Slender, flexuous, branching from near the base: root long, at first filiform at length lignescent: stem erect or ascending, finely appressed-pubescent: earliest leaves 1–5-foliolate, the later ones 7–19-foliolate: leaflets very variable, oblong, retuse, or in var. **angustissima** (*T. angustissima* Shuttlew.) linear and acute; stipules filiform; flowers small, purple, the lowest pair usually axillary, the others forming a long slender erect raceme: pods spreading or nodding, 4^{mm} broad, appressed-pubescent under a lens, 3–5-seeded.—Syn. 2:

329; Hook f. & Jacks. Ind. Kew. **2**: 1045. *T. leptostachya* DC. Prodr. **2**: 251. 1825; Chapm. Fl. ed. 2, 616. *T. adscendens* Macfad. Fl. Jam. 257. *T. tenella* Gray, Pl. Wright. **2**: 36. *T. angustissima* Shuttlew. in Chapm. l. c. 96. *Cracca purpurea* L. Spec. **2**: 752; Vail, l. c. 31. *Galega piscatoria* Ait. Kew. **3**: 71. —Sandy ridges, Florida, *Rugel, Curtiss*, no. 584*, *Garber, Simpson*, also from W. Texas, *Havard*, to Arizona. (Tropics of both hemispheres).

GRAY HERBARIUM.

BRIEFER ARTICLES.

NOTES OF TRAVEL. II.

PAYTA AND THE DESERT REGION OF PERU.

When Mr. Barbour Lathrop, with whom the writer is traveling as botanical assistant, first decided to go *via* Panama to the west coast of South America, he remarked that he would show him Payta, the driest, most forsaken spot in the world. He would defy even a botanist to find so much as a single living wild plant. The donkeys of Payta are reputed, like the locusts during early days in Kansas, to eat any green paint in sight.

Payta lies less than five degrees south of the equator in the dry zone of Peru, on a coast, steadily rising from the sea in some parts, which has risen as much as forty feet within historic times. So infrequent are the rains on this coast that, when they do come, the whole native population, with crucifixes and musical instruments, goes out to welcome the river as it slowly forces its way along the bed which for seven years has been as dry as the surrounding desert. This coming of the river indicates heavy rains on the west slope of the Andes and is generally followed by showers in the region about Payta.

When we left Panama it was rumored there had been rain at Payta. Somewhat to Mr. Lathrop's disappointment, these rumors were verified when we anchored off the coast, and with the glasses discovered in one of the small valleys a green algaroba shrub (Prosopis). By looking closely we saw an almost imperceptible film of green spread over the tops of the light brownish-gray hills. On landing, we discovered that until February 9, when it rained from 10:00 P. M. until the following noon, no rain had fallen for eight years. The seventh year had failed to bring the usual periodic rains and the people were greatly alarmed lest another seven years should pass without water for the cotton crop upon which they depend largely for their support. The Piura river bed and a strip of land on each side which is overflowed form, after the subsidence of the stream, which runs only a month or two, the cultivated land of the country back of Payta. The long

rooted Peruvian cotton is able to maintain itself for seven years in this dried-out river bed and yields profitable crops of the colored, short staple cotton, which is used as an adulterant for wool, occupying a place in the wool rather than the cotton market. A stroll to the top of the nearest hill at Payta showed plainly that the rain had been a heavy one, for, scattered over the nearly level table land were the hard baked remains of unmistakable mud puddles. In these, strange as it

FIG. 1.—Photograph of garden at Mollendo, produced by irrigation.

seemed to us, no plants were found, although scattered over the sand and gravel all about were young seedlings and even blooming grasses.

The flora of Payta would not be a difficult one to write up exhaustively, provided one were on the spot at the right time. In a small pamphlet we were able to press all the phanerogamous plants which were found, without any difficulty. These plants comprise annuals whose seeds must have remained dormant since the last rain, eight years before, and perennials which have kept alive by encasing their

tissues in thick layers of impermeable cork. There is something remarkable in the ability which these desert shrubs have of reducing their transpiration surface to such a degree that they can withstand the intense insolation of this tropical region, and the even more trying influence of an extremely dry atmosphere. It is highly probable that they are able, during the winter season, to absorb moisture from the fogs which are blown in from the ocean. Owing to the cool currents

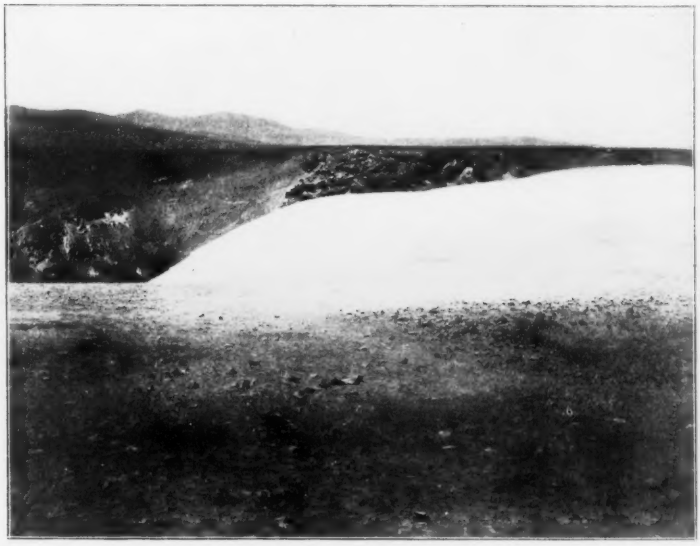


FIG. 2.—Photograph taken just outside of garden fence, showing the completely barren desert.

of air which blow summer and winter across these deserts, they are not exposed to such temperatures as would be expected in this latitude.

The collection of plants—the provisional flora of Payta—consists of two perennials, a Prosopis and an undetermined shrub, and seven annuals, three grasses, a lupine, a caryophyll, a seedling amaranth, and a beautiful yellow flowered oxalis. Our visit to Payta was twenty-two days after the rain, and the grasses and oxalis were in full bloom. The sight of hundreds of delicate yellow blossoms scattered over the perfectly barren hillsides, and slender blades of grass so far apart that

they looked like a very "bad catch" at lawn seeding, is one which few who have not been in the deserts in spring can imagine. Unlike the desert regions of our own country, with their sage brush, yuccas, and host of small tufts of grass and sedge, these deserts are for miles absolutely without a living plant. For days we steamed down the coast, but aside from an occasional garden made by irrigation in the neighborhood of the towns, we saw no green plant of any description. From about Payta in Peru, to Carrizal in Chili, representing fourteen days of steamer travel, the coast presents one unbroken line of desert. At Arica it is broken by a small fertile valley, and at Carrizal the desert ends in a scanty vegetation of cacti and low growing cushion-like perennials.

At Mollendo we had an opportunity to see what this desert might have been had there been an abundance of rain. The two photographs are taken within a hundred meters of each other. One shows a private garden in Mollendo with apples, peaches, grapes, passion fruits, figs; in short a good collection of fruit and shade trees. The other is a representative view of the surrounding country as barren as a fresh lava bed.

There are below this desert at Arica, and doubtless at other points, underground sources of water, for large pepper trees which have been planted in the town square are growing as finely as they do in Gibraltar, or southern California, and overshadowing the little clubhouse at Arica is one of the largest fig trees I have ever seen.

There is no place in the world under the tropics which possesses such a cool agreeable climate as this "zona sicca," or dry zone of western South America. The contrast between the west and the east coasts of the continent at the same latitude is very remarkable. At 5° S. off Brazil, duck suits are necessary for comfort, while at the same latitude off Peru thin flannels are quite comfortable.

For a systematic botanist, as may be judged from the above description, there is not much of interest in this region, but from a physiological point of view it will yield some very interesting facts.

Dr. Schimper, in his *Pflanzengeographie* p. 679, calls attention to the fact that this desert region of Peru has been very little studied from an ecological standpoint. It is probable that a very small number of species will be found along this coast, and hundreds of square miles are absolutely without a living plant for years at a time. Certain localities, however, favored by the fogs, are covered in the winter

season (our summer) by grasses in sufficient quantities to support numerous small herds of cattle.

The most favorable point from which to begin an ecological study of this desert region would be Payta and the inland town Piura behind it, which can be reached by railway. Having made arrangements at Piura for mules and a guide, the towns of Pacasmayo and Salaverry would not be too far apart to serve as centers for operation down the coast. The discomforts of travel through this desert, I understand, are not such as should deter any enterprising botanist from exploring it. The expenses, including steamboat travel for which the charges are twenty pounds sterling from Panama to Callao, would be easily within five dollars a day. — DAVID G. FAIRCHILD.

SECTION G (BOTANY), A. A. A. S., COLUMBUS MEETING.

THE meeting of section G began on Monday, August 21, in Townshend Hall of the Ohio State University, by a brief session for organization. In the afternoon at four o'clock, in Botanical Hall, the vice president, DR. CHARLES REID BARNES, delivered an address on *The progress and problems of plant physiology*. At the close of the address, the thanks of the section were voted to the speaker.

The reading of papers began on Tuesday, when the following were presented in full, or in abstract, or by title:

F. L. STEVENS: The fertilization of *Albugo bliti*.

FRANCIS RAMALEY: The embryo sac of *Leucocrinum montanum*.

A. S. HITCHCOCK: Notes on subterranean organs.

W. J. BEAL: Some monstrosities in spikelets of Eragostis and Setaria, with their meaning.

CHARLES E. BESSEY: Studies of vegetation of the high Nebraska plains.

A. D. SELBY: The tamarack swamp in Ohio.

WM. SAUNDERS: The breeding of apples for the northwest plains.

BYRON D. HALSTED: Field experiments with "nitragin" and other germ fertilizers.

HENRY L. BOLLEY: The duration of bacterial existence under trial environments.

Wednesday was designated *Sullivant Day*, and was used to commemorate Wm. S. Sullivant (died 1873) and Leo Lesquereux (died 1888), two most able bryologists who were long residents of Columbus. Through the initative and energy of Mrs. E. G. Britton and the

assistance of a number of botanists, an exhibition of many interesting bryological books and pamphlets, type specimens and original drawings of mosses, photographs, portraits, and autographs of bryologists, maps of distribution, etc., was given during the day in the large lecture room in Botanical Hall, where Wednesday's sessions were held. Portraits of Sullivant and Lesquereux, loaned by their daughters, formed the center of interest. This exhibition attracted much attention and was warmly commended.

The exercises in honor of Sullivant and Lesquereux were as follows:

Professor W. A. Kellerman read a portion of Gray's tribute to Sullivant.

Professor C. R. Barnes read a biography of Lesquereux.

Mrs. E. G. Britton gave a brief account of the species of mosses named for Sullivant.

A letter was read from Professor Arthur Hollick regarding the paleobotanical work of Lesquereux.

Professor L. M. Underwood gave a brief outline of the progress in the study of the Hepaticæ of North America, and Mrs. Britton for the Musci. Both addresses were illustrated by the books, pamphlets, photographs and maps of the exhibition.

Professor F. E. Lloyd exhibited the plates and type specimens of the twelve new species of liverworts described by Dr. M. A. Howe in his recent monograph.

Professor W. A. Kellerman presented to each member of the section a set of six species associated with the names of Sullivant and Riddell, an early Ohio botanist. The specimens were from type localities in most cases. They were *Sullivantia Sullivantii*, *Lonicera Sullivantii*, *Arabis patens*, *Solidago Ohionis*, *S. Riddellii*, and *Trillium nivale*.

Mrs. E. G. Britton also distributed specimens of *Orthotrichum Ohioense* and *Bryoxiphium Norvegicum*.

The following papers were also read:

CHARLES MOHR: Notes on the moss flora of Alabama.

A. J. GROUT: Suggestions for a more satisfactory classification of the pleurocarpous mosses.

BRUCE FINK: Notes concerning the study of lichen distribution in the Mississippi valley.

W. C. STEVENS: Botanical teaching in the secondary schools.

IDA CLENDENIN: Botanical teaching in the secondary schools.

The two papers on botanical teaching had been prepared by request and evoked an interesting discussion.

On Thursday the following papers were read:

H. A. HARDING: On the occurrence of the black rot of cabbage in Europe.

CHARLES E. BESSEY: One thousand miles for a fern.

WALTER T. SWINGLE: A summary of our knowledge of the fig.

WM. TRELEASE: The classification of botanical publications.

As a result of the discussion of this paper, transmitted by the Botanical Society of America, the following resolution was adopted:

Resolved, That Section G recommends as a basis for the classification of a botanical library the decimal system now in common use in the United States. The section requests that the suggestions embodied in Dr. Trelease's scheme for classification be adapted, so far as possible, to that system, and that the paper be then published in *Science* for the purpose of eliciting discussion.

EDWIN B. COPELAND: The geotropism of the hypocotyl of cucurbits.

A. F. WOODS: The destruction of chlorophyll by oxidizing enzymes.

Apropos of this paper the following action was taken:

Resolved, That Section G express its gratification at the appointment of an eminent physiological chemist to the staff of the Division of Vegetable Physiology and Pathology of the Department of Agriculture.

The secretary was instructed to communicate this resolution to the Secretary of Agriculture.

C. O. TOWNSEND: The effect of hydrocyanic acid gas upon the germination of seeds.

W. G. JOHNSON: Some physiological effects of hydrocyanic acid gas upon plants.

A communication was read from the Cambridge Botanical Supply Co., which has been publishing the card edition of the bibliography of American botany under the direction of the Section's Committee on Bibliography, stating that the publishers must terminate the present arrangement at the close of 1899 on account of the fact that the number of subscribers was insufficient to meet the cost of publication. After discussion the committee was increased to five by the appointment of D. T. MacDougal, of New York Botanical Garden, and J. F. Cowell, director of the Buffalo Botanical Garden. The section expressed its approval of the card index and empowered the committee to arrange for its continuance in any feasible way.

On Friday the following papers were read:

J. C. ARTHUR: The cultures of Uredineæ in 1899.

FRANCIS E. LLOYD: The embryology of *Vaillantia hispida*.

A. D. SELBY: The flora of Franklin county, Ohio.

ERWIN F. SMITH: The fungous infestations of agricultural soils in the United States.

C. E. BESSEY: Are the trees advancing or retreating upon the Nebraska plains?

WM. SAUNDERS: Useful trees and shrubs for the northwest plains of Canada.

H. L. BOLLEY and L. R. WALDRON: The occurrence of calcium oxalate and lignin during the differentiation of the buds of *Prunus Americana*.

HERMANN VON SCHRENK: Two diseases of Juniperus.

WM. B. STEWART: Etiolative reactions of Sarracenia and Oxalis.

JULIA B. CLIFFORD: The mycorhiza of Tipularia.

HENRY KRAEMER: The crystals in *Datura Stramonium*.

At 3 P.M. on Friday the section adjourned, *sine die*.—C. R. B.

BOTANICAL SOCIETY OF AMERICA.

THE fifth annual meeting was held in Columbus, Ohio, August 18, 19, under the presidency of DR. LUCIEN MARCUS UNDERWOOD. All sessions were held in buildings of the Ohio State University. The address of the retiring president, DR. NATHANIEL LORD BRITTON, upon *The development of the New York Botanical Garden* was given in the chapel of University Hall on Friday evening. It was copiously illustrated with lantern slides, showing the original site, with its natural beauties, the progress of the planting, buildings, etc. The address will be published in full in a later number of the GAZETTE. At the close of the address the thanks of the audience were voted to the speaker for the interesting and able presentation of facts regarding the great institution of which he is director. The following papers were read in full or in abstract or by title before the society. Abstracts of some of them will be found in later pages.

CHARLES E. BESSEY: *Apetaly and diœciousness.*

BRADLEY M. DAVIS: *The spore-mother-cells of Anthoceros.*

DANIEL T. MACDOUGAL: *Symbiosis and saprophytism.*

DAVID M. MOTTIER: *The effect of centrifugal force upon the cell.*

NATHANIEL L. BRITTON: *The American species of Arisæma.*

JOSEPH C. ARTHUR: *The Uredineæ occurring upon Phragmites, Spartina, and Arundinaria in America.*

BYRON D. HALSTED: *Distribution of American Erysipheæ.*

BRADLEY M. DAVIS: *Gametes and gametangia of the Phycomycetes.*

WILLIAM TRELEASE: *Classification of botanical publications.* The society requested that this paper be read also before section G, A. A. A. S.

DANIEL T. MACDOUGAL: *Etiolative reactions.*

LUCIEN M. UNDERWOOD: *The foundations of genera in ferns.*

The officers elected for 1900 are: BENJAMIN LINCOLN ROBINSON, *president;* BYRON DAVID HALSTED, *vice president;* ARTHUR HOLLICK, *treasurer;* GEORGE FRANCIS ATKINSON, *secretary;* B. T. GALLOWAY and D. P. PENHALLOW, *councillors.*

The new members elected were: J. M. Macoun, Geological Survey, Ottawa, Can.; W. J. Beal, Agricultural College, Mich.; C. F. Millspaugh, Field Columbian Museum, Chicago; Marshall A. Howe, Columbia University, New York.

Amendments to the constitution were adopted, creating as additional classes of members, life members, associates, and patrons. The new sections are as follows:

Section 3. Life Members.—Any member of the Society may become a Life Member by the payment to the treasurer of one hundred dollars at any one time. Life membership fees shall be added to the permanent fund of the Society.

Section 4. Associates.—Associates of the Society may be elected in the manner already prescribed for members. Before the first of January following his election, each Associate shall pay into the treasury of the Society annual dues to the amount of five dollars. Associates shall have all the privileges of members except that of voting, and of holding office. Subsequent to the adoption of this provision members shall be chosen only from the list of associates.

Section 5. Patrons.—The payment to the treasurer of a sum not less than two hundred and fifty dollars at any one time, or a bequest of such sum, shall constitute the donor a Patron of the Society. The names of Patrons shall be published with the annual lists of officers and members, and the Patrons shall be entitled to receive copies of all the publications of the Society. Patrons' fees shall be added to the permanent fund of the Society.

The report of the treasurer shows funds on hand amounting to $1438.27, of which $1260 is on deposit in savings banks. A committee was appointed to invest funds where a higher rate of interest may be obtained than from savings banks. The total assets of the Society are now $1517.59.—C. R. B.

BOTANICAL CLUB, A. A. A. S.

UNDER the presidency of Dr. Byron B. Halsted, with Professor A. D. Selby acting as secretary, the Botanical Club listened to the following brief communications:

C. E. BESSEY: A greasewood compass plant.

C. E. BESSEY: A visit to the original station of the Rydberg cottonwood.

N. L. BRITTON: Report on Mr. Heller's botanical exploration of Porto Rico.

F. S. EARLE: Tomato fruit rot.

W. J. BEAL: The botanical club of the Michigan Agricultural College.

WM. SAUNDERS: The arboretum and botanic garden of the Central Experimental Farms, Ottawa, Canada.

F. E. LLOYD: On two hitherto confused species of Lycopodium.

L. M. UNDERWOOD: What shall we regard as generic types?

L. C. CORBETT: A device for registering plant growth.

A. D. SELBY: The introduced species of Lactuca in Ohio.

O. F. COOK: Notes on some of the work of the Division of Botany of the U. S. Department of Agriculture.

THOMAS A. WILLIAMS: Some features of the investigations on grasses and forage plants, in charge of the Division of Agrostology, U. S. Department of Agriculture.

J. W. T. DUVEL: A brief embryological study of *Lactuca Scariola* L.

N. L. BRITTON: Notes on the northern species of Celtis.

N. L. BRITTON: Remarks on some species of Quercus.

W. J. BEAL: The introduction of *Cabomba Caroliniana* in Michigan.

C. E. BESSEY: The wilting of *Cleome integrifolia.*

W. A. KELLERMAN: Labels for living plants.

H. L. BOLLEY: The position of the fungi in the plant system as indicated by the work on the organisms of nitrification.

L. M. UNDERWOOD: Summary of our knowledge of the distribution of fungi in America.

C. E. BESSEY: The powdery mildew of *Polygonum aviculare.*

A. D. SELBY: On *Plasmopara Cubensis.*

A. S. HITCHCOCK: Distribution of some Kansas plants.

W. R. LAZENBY: Unusual development of leaves and growth of plants from cuttings.

A. D. HOPKINS: Some botanical notes by an entomologist.

A. S. HITCHCOCK: Some wheat crosses.

On Thursday morning the Club adjourned *sine die.*

THE SEXUALITY OF THE TILOPTERIDACEÆ.[1]

Ectocarpus pusillus Griffiths or *Acinetospora pusilla* Bornet possesses two forms of reproductive organs, the unilocular and plurilocular sporangia, whose elements present conditions intermediate between zoospores and aplanospores. I have recently encountered a third form of reproductive organ, the monosporangium, identical with that of *Haplospora Vidovichii* Bornet or *Heterospora Vidovichii* Kuckuck, and this last species ought henceforth to be placed in the genus Acinetospora.

These monospores are uninucleate, like the so-called oospheres of *Scaphospora speciosa* (said to be the sexual form of *Haplospora globosa*), and are covered by a membrane derived from the interior of the monosporangium, as are likewise the 4-nucleate monospores of *Haplospora globosa*. Since they possess a membrane before their dehiscence they cannot be fertilized. On account of the frothy structure of their protoplasm and the great variation in their dimensions, they resemble neither oospheres nor spores. They germinate readily in cultures and develop little plants that bear the same kind of reproductive organs. I regard them as *gemmae* or *propagula*, and the organ that contains them is a pseudosporangium. From what we know of the Cutleriaceæ and Ectocarpus it is possible that the plurilocular sporangia of *Acinetospora pusilla* are female organs whose oospheres germinate parthenogenetically. The antheridia will undoubtedly be found some day.

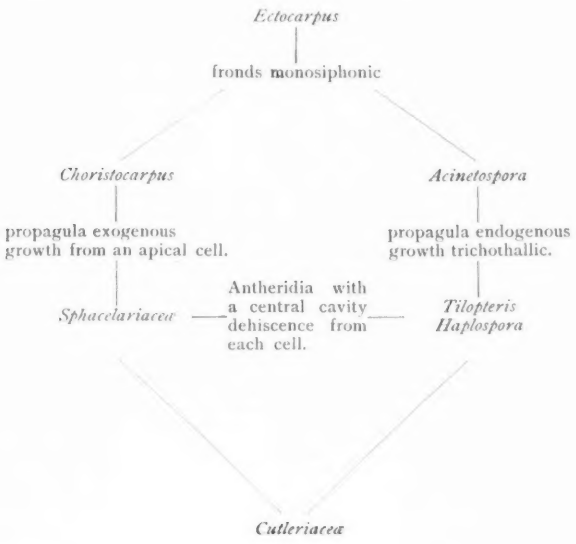
The same conclusion as to the nature of the monospores is justified for Tilopteris and Haplospora. We are no longer able to admit that these plants possess spores and oospheres similarly situated, of like form and similar dimensions, with the same protoplasmic structure and identical methods of germination, but that the spores germinate normally while the oospheres develop parthenogenetically. It seems more satisfactory to say that one or the other of the elements are propagula and that the 4-nucleate propagula are those that have begun to germinate upon the mother plant.

We shall say then that according to the present state of our knowledge the early known representatives of the Tilopteridaceæ, Tilopteris, and Haplospora propagate themselves solely vegetatively. The antheridia of Tilopteris give rise to true antherozoids that really seem to be rudimentary structures, but it is by no means certain that they

[1] This contribution is a *résumé* by the author of the paper entitled "Les Acinetospora et la sexualité des Tilopteridacées," Jour. de Bot. **13**: —. 1899. Translated by Dr. Bradley M. Davis.

are the same as those organs of Haplospora, however similar the type. We do not know of female organs, but we may foresee that they would have the form of plurilocular sporangia with a median cavity and a separate dehiscence for each little cell. Similarly one may foresee that the antheridia of Acinetospora will be provided with a median cavity and that the dehiscence will be common and terminal.

The Tilopteridaceæ have no affinity with the Fucaceæ with which they have often been placed in the classical works. On the contrary they approach very closely the Ectocarpaceæ and Cutleriaceæ. They may be divided into two tribes, the ACINETOSPOREÆ (genus Acinetospora), more closely related to the first; and the HAPLOSPOREÆ (genera Tilopteris and Haplospora), more closely related to the second. When these plants shall show us organs that are as yet unknown we shall without doubt have to raise the preceding tribes to the rank of families, the Acinetosporaceæ and Haplosporaceæ. I have shown in the table below a way in which the affinities of these plants may be represented.—CAMILLE SAUVAGEAU, *Dijon, France.*



FLOWER VISITS OF OLIGOTROPIC BEES.

SINCE the proof of my last paper in this journal was read I have had occasion to doubt the correctness of the statements regarding Epeolus, on pages 35–37, and have asked Mr. Ashmead if he still held the views credited to him. In a letter of August 2, he writes that the nests were evidently made by *Entechnia taurea* and that the Epeolus was merely entering them, not making them, as he supposed at the time. Epeolus, therefore, comes under the same category as Nomada. After all, the phenological position of the genus corresponds pretty well with that of Melissodes upon which most of the species are probably inquiline.

In the table of oligotropic bees *Xenoglossa cucurbitarum* should be included. Lately I have found it collecting pollen of *Cucurbita pepo*. It also visits *Citrullus vulgaris*, *Asclepias Cornuti*, *Ipomœa nil* and *I. pandurata*. It has been taken at Ames, Iowa, by Miss Alice M. Beach, on flowers of "summer squash;" at Mesilla, New Mexico, by Mr. Cockerell, on flowers of *Cucurbita perennis;* at Metropolis, Ill., by Mr. Hart, ou *Martynia proboscidea*, and is mentioned in the GAZETTE (**17**: 65) under the MS. name *X. brevicornis*. I suspect that all of our species of Xenoglossa get their pollen exclusively from Cucurbitaceæ. —CHARLES ROBERTSON, *Carlinville, Ills.*

QUERCUS ELLIPSOIDALIS IN IOWA.

MR. WILLIAM D. BARNES, of Morgan Park, Illinois, has placed in my hands specimens of an undetermined oak, collected by him in 1895 at Big Rock, Scott county, Iowa, which proves to be *Q. ellipsoidalis* Hill. Mr. Barnes and two collaborators are preparing a catalogue of the plants of Scott and Muscatine counties for the Davenport Academy of Sciences, but have been unable to determine the name of this oak. The field note accompanying this specimen reads: "Tree with smooth bark, and with the general aspect of *Q. palustris*." It is a fruiting branch, the leaves quite small and rather narrower than those commonly found near Chicago, but such as may frequently be seen on individual branches, or as to size may characterize nearly an entire tree. The acorn is one of the smaller kind, closely resembling the one figured in *Plate II, e*, BOTANICAL GAZETTE, March 1899.—E. J. HILL, *Chicago.*

A NEWLY OBSERVED STATION FOR *GALINSOGA HISPIDA*.

SOME time ago the writer[2] called attention to the fact that besides *Galinsoga parviflora* Cav. and its var. *hispida* DC., another quite distinct species, *G. hispida* Benth., had been found in the Atlantic states, occurring at Camden, New Jersey, where it was collected on waste lands by Mr. C. F. Parker. This species was again found last October by Mr. Howard Schriver at several points in Cumberland, Maryland. Mr. Schriver, noticing its differences from *G. parviflora* Cav., sent specimens to the U. S. National Museum, whence they were kindly forwarded to the writer by Dr. J. N. Rose.

Mr. Schriver reports the plant as not only forming a large mass of vegetation (5 or 6 meters long) near a "bonded warehouse" or sort of cattle depot, but also extending down the banks of an adjacent stream where individuals were scattered on steep cliffy slopes of Helderberg limestone, even reaching the water's edge. A sporadic specimen was also found on a country road not far away. While the presence in the neighborhood of the cattle yard, a railway, and a distillery would readily account for the introduction of plants from a distance, the present species in the luxuriance of its growth and tendency to spread into the indigenous vegetation would suggest that it has found congenial conditions and is likely to persist. The species is readily distinguished by its "pink" or "red" rays (drying purple) and by it short pappus, which is about half the length of the achene. In foliage and rather copious spreading pubescence it resembles *G. parviflora*, var. *hispida* DC., but both *G. parviflora* and its variety have white rays and much longer pappus (nearly or quite the length of the achene).

As *G. hispida* Benth. is likely to be found at other stations in the middle Atlantic states it may be worth while to cite its more important synomymy, which is as follows:

Vargasia caracasana DC. Prodr. **5**: 676. 1836.

Galinsoga hispida Benth. Bot. Sulph. 119. 1844.

G. brachystephana Regel, Ind. Sem. Hort. Turic. 2. 1846.

G. caracasana Sch. Bip. Linnaea **34**: 529; also Bull. Soc. Bot. Fr. **18**: 86. 1865.—B. L. ROBINSON, *Gray Herbarium*.

[2] Proc. Am. Acad. **29**: 326.

CURRENT LITERATURE.

BOOK REVIEWS.

Engler and Prantl's Pflanzenfamilien.

THIS great work has been noticed from time to time in the BOTANICAL GAZETTE as the various parts have appeared. But now that Volumes II–IV are complete, which contain the siphonogams, the time seems appropriate for a more extended notice. The first part appeared in 1887, and twelve years later the three volumes of siphonogams were finished. The publication of the three volumes of Bentham and Hooker's *Genera Plantarum*, covering the same ground, but with no such breadth of treatment, extended from 1862–1883, a period of twenty-one years. There is no definite statement as to the completion of Volume I, devoted to cryptogams, but several sections of it have been published, and other parts are appearing with reasonable rapidity.

So far as statistics are concerned, it may be of interest to note that, excluding the indexes and the cryptogams, there are twenty-six sections of the work, each with its separate index, and forming handy laboratory volumes. The number of genera treated by the fifty-seven collaborators is 8218. The pages are 6997 in number, the original illustrations 3026 (woodcuts 3023, heliogravures 3), and the individual figures 19,366. The total price is *M* 436, or bound in eleven half morocco volumes *M* 474.50.

When one considers such details he is impressed by the magnitude of the work, and still more by the organizing power which has kept the large plans in operation through so many years. The editorial work must have been enormous, to bring contributions necessarily heterogeneous into a reasonable degree of uniformity. But while the details are impressive they do not indicate the importance of the work. That it marks an epoch in taxonomic publications does not need to be stated as a prophecy, for during its publication it has achieved this distinction. Lists, manuals, and herbaria were using the Engler and Prantl sequence long before the work was complete. This was due to the fact that it sought to relate plant groups upon the basis of what is known concerning them, and discarded the old groupings which had long been the laughing stock of biologists. In other words, this great work breathes into taxonomy the modern biological spirit, and makes it more than a set of names.

Superior to all previous general works in its spirit, it is alone in the number and beauty of its illustrations. Every family is thoroughly and

admirably illustrated, and we venture the prediction that many of these figures will become classic in future texts. No such collection of figures representing the plant kingdom exists, and they give a conception of plants in general that can be obtained from no other publication. The figures and text include not merely those structures which may be said to have taxonomic importance, but anatomical peculiarities of each family are set forth. All through the work the ecological standpoint is prominent, and the sections on geographical distribution are among the most valuable.

It is to be expected that the treatment is unequal, and the different parts of very different degrees of merit, but with fifty-seven collaborators this could not be avoided. It seems to most botanists far more important to complete a work within a reasonable time, and so establish a usable datum-line, than to drag it out indefinitely and allow one part to be out of date before another is published. In general the treatment will be regarded as conservative, there being apparent no desire for change if existing lines can be used at all. In so delicate a matter as nomenclature, as is well known, the "Berlin rules," which are in fact the Engler rules, are drawn up in the spirit of compromise, not going to either extreme, and probably satisfying neither set of extremists. No set of rules proposed, however, has had as yet such a tremendous advantage of general usage as this great work will compel for the Berlin rules.

It is impossible to mention in detail the views advanced as to the evolution of plant groups. There will be much difference of opinion as to minor points, for many smaller groups, through lack of adequate investigation, had to be "lumped," but in the judgment of the reviewer the main lines of evolution suggested will stand, which are in brief as follows: spiral arrangement and indefinite numbers to cyclic arrangement and definite numbers; naked flowers to differentiation of calyx and corolla; apocarpy to syncarpy; polypetaly to sympetaly; hypogyny to epigyny; actinomorphy to zygomorphy. That cases of "reduced flowers" occur there can be no doubt, but that the great majority of so-called cases of reduction are really primitive in character seems hardly less doubtful. — J. M. C.

Ferments and fermentation.

THE attention which the various problems connected with fermentation have received during the past decade and the interest, both theoretical and practical, which attaches to the investigation of these problems make doubly welcome a book on the soluble ferments from the hand of Professor J. Reynolds Green.[1] In it he has sought to bring together, so far as possible, the results already reached, and to indicate the view of the processes of

[1] GREEN, J. REYNOLDS: The soluble ferments and fermentation. 8vo. pp. xiv + 480. Cambridge: The University Press. 1899. 12 s. [New York: The Macmillan Company.]

fermentation to which these results point. For it can hardly be said that the results now attained furnish any adequate explanation of fermentation; though they remove it more completely from the realm of so-called "vital" action, they refer it to the category of equally inexplicable catalytic phenomena.

After discussing the nature of fermentation and its relation to enzymes, the author gives a detailed account of diastase (60 pp.), inulase, cytase, sugar-splitting enzymes, glucoside-splitting enzymes, proteolytic enzymes (57 pp.), fat-splitting enzymes, clotting enzymes (48 pp.), ammoniacal fermentation, oxidases, alcoholic fermentation, and the fermentative power of protoplasm. The work closes with chapters on the secretion, constitution, and mode of action of enzymes. Of course the treatment of these topics includes a discussion of the discovery, occurrence, preparation, and behavior of enzymes in both animal and plant bodies.

A work like this is not open to adverse criticism. It is rather to be commended without stint. Indeed, every physiologist will be thankful to know and to have at hand this compact but full summary of researches, accompanied, as it is, by an extensive bibliography, leading to further details in the original papers. One notices with pleasure that even the results contradictory to the general trend of investigation are clearly stated. This engenders confidence in the fairness of the work; a confidence which the closest scrutiny justifies.

The bibliography, which the author modestly says is not exhaustive, is especially to be commended. Its extent will be appreciated when it is stated that it contains about 800 titles! The citations would have been improved had some uniform system been followed. Here is an assortment of five styles taken from p. 449: (1) Zeit. f. Biologie Bd. x. 92; (2) Zeitsch. f. klin. Med. 1. (1880). 231; (3) Cent. f. Bact. 1891. Bd. 10. 401; (4) Cent. f. Bakt. 15 (1894). 722; (5) Ber. d. deut. chem. Gesell. 23 (1890). 3689; (6) Ber. d. deut. chem. Gesell. (1895), 1433. This will be looked upon by many as of small consequence; and so it is, in comparison with the value of the work. But the defect is so unnecessary and so easily avoided that it seems the greater pity that it should mar so good a bibliography. Moreover, calling attention to it here may serve to emphasize a point which many scientific writers sorely need to lay to heart.

Users of this work will be thankful that the editor of the *Cambridge Natural Science Manuals*, of which this is one, had an index prepared, in spite of the author's insistence that one was unnecessary. They will only be sorry that the index is not fuller, and that it does not include the names of authors, for which the bibliography must be consulted.—C. R B

NOTES FOR STUDENTS.

BEFORE THE Botanical Society of America, at the Columbus meeting Professor D. T. MacDougal read a paper on *Symbiosis and saprophytism*, of which the following is a synopsis:

At the last meeting of the society I read a paper in description of my work upon a large number of herbaceous mycorhizal plants,[2] and a short note was presented before the meeting of the Society for Plant Physiology and Morphology, in December 1898, in which a delimitation of the terms saprophyte and symbiosis was attempted. Attention was also called to the fact that only two seed plants, Wullschlægelia and Cephalanthera, may be truly designated as saprophytes, all other species of so-called holosaprophytes being symbiotic with mycorhizal fungi.

During the last year my efforts have been directed first to ascertain the adaptations undergone by these true saprophytes, and compare such changes with those undergone by mycorhizal forms. Secondly, evidence which might have a bearing upon the physiological relations of a seed plant and its mycorhizal fungus has been carefully sought for.

Cephalanthera, the saprophyte examined, showed alterations in structure generally similar to those of mycorhizal forms; but, in exception to the majority of chlorophylless species, it retains the stomata of the leaves, and has developed no underground transpiratory organs.

The roots, unlike those of most mycorhizal forms, are deeply buried in the soil, on account of which the number of good herbarium specimens to be found is extremely small. Two types of these organs are present: a fibrotype, with a reduction and fusion of the stelar components, and with radially elongated cortex. This variation has hitherto been regarded as a result of the presence of a fungus as in mycorhiza, while as a matter of fact it is an adaptation to humus foods. The second type of root is devoted to storage and has a normal multiplication of the cortical elements. Both types agree closely with the two kinds of roots formed by Wullschlægelia, which still further strengthens the conclusions given above.

The lack of apparatus for the conversion of radiant energy indicates that these two saprophytes must receive their entire supply from the chemical energy of the humus compounds taken up, and we may decide inferentially that substantial modifications of the minute mechanism of absorption must have taken place, entailing also unusual osmotic conditions. In general these two seed saprophytes live like fungi, although capable of forming starch.

The coralloid formations on the offsets of Calypso, which are found from Norway to Washington, have been examined, and the result of the development of these adventitious mycorhizas is quite similar to that already described in Aplectrum. The occurrence of the coralloid mycorhiza is coupled

[2] Published in the *Annals of Botany* 13: —. March 1899.

with a diminution of the leaves, the roots, and the storage organs, indicating an increased acquisition of highly organized food-material; a variation which will have the same ultimate influence on the species as in Aplectrum, and is doubtless responsible for many of the so-called aberrant forms of leaves and flowers reported.

This species and *Corallorhiza Arizonica* were examined with especial reference to the relations of the seed plant and the endotropic fungus. *Corallorhiza Arizonica* is entirely free from chlorophyll, and the aerial shoot has lost the stomata. The subterranean rhizome and its coralloid branches are each furnished with separate types of motile stomata, however, which function during the entire year, but clearly serve transpiratory and respiratory needs alone.

The arrangement of the fungus is one which has been found in nearly all of the endotropic mycorhizas hitherto examined, and its study has yielded some important results.

The hyphæ of the fungus extend themselves in comparatively straight threads longitudinally toward the tip of the organ in the sub-epidermal region of the cortex, and this portion remains alive even in old members constituting what may be designated as the "vegetative mycelium." The entrance of the hyphæ of the vegetative mycelium into the young cortical cells causes almost no disturbance in the character of the latter, and no important interchange between the two plants ensues in this region.

As the growth of the root and the contained vegetative mycelium goes on, the younger portion of the mycelium sends out hyphal branches which extend out through the epidermis and may or may not traverse the hairs into the humus soil; these are very clearly absorbing organs. At the same time another set of branches penetrate to the median region of the cortex and both set up and undergo profound disturbances. The tips of the hyphæ are attracted to the vicinity of the nuclei. Dense coils and sometimes large vesicles are formed which serve as organs of interchange between the fungus and the seed plant. The starch contained in the cortical cells inhabited by these organs is used by the fungus; then, with the maturity of this general region, the organs of interchange and the large amount of contained proteid are set free and become available to the higher plant.

Here then is the character of the partnership of the two plants:

The seed plant furnishes a habitat for the vegetative mycelium of the fungus, and yields to it certain carbohydrate foods, principally starch. The fungus takes up the humus products from the soil by means of its external branches, conducts them to the inner branches in the cortex of the higher plant, and manufactures proteids, of which a portion is used in its own metabolism of course, but the greater part is yielded to the seed plant.

It follows as a necessary corollary from the above conclusions, that Frank's theory that mycorhizal adaptations are fungus traps and that the seed plant

derives the entire advantage from the association is no longer tenable. Likewise the theory of Janse, that endotropic fungi which form mycorhizas are negatively chemotropic to oxygen and bear the same relation to the seed plant as the organism in the leguminous tubercle, is not capable of universal application. Such relation has been proven by Nobbe and Hiltner between Podocarpus and the peronosporous fungus which forms endotropic mycorhiza with it, but in no other instance.

At this stage of our investigations, then, two distinct physiological types of endotropic mycorhizas are recognized: One adapted for nitrogen fixation, and a second for the absorption and modification, perhaps oxidation, of humus products by the fungus and their liberation in the tissues of the higher plant. The greater number of examples are included under the last type.

Ectotropic or sheathing mycorhizas such as Monotropa perhaps approximate more nearly to the latter type.[3]

THE PAPER read before the Botanical Society of America, at Columbus, entitled "The effect of centrifugal force upon the cell," by Professor D. M. Mottier, discussed in detail the effect of centrifugal force varying from 1800 to 1900 times that of gravity, acting for definite short periods of time, upon cells of certain algæ, mosses, and phanerogams. In all cells operated with, the movable plasmic contents, together with the inclusions, were made to fall into a compact mass at the end of the cell. In cells which were not killed outright or too badly injured, so that death resulted soon afterwards, the displaced cell-contents gradually redistributed themselves in due course of time.

The most strikingly interesting phenomena are presented by dividing cells and in the behavior of the nucleolus. In dividing cells of such algæ as Cladophora and Spirogyra, the cell-wall in process of formation at the time of centrifugal action was never completed. The chloroplasts, nuclei, and other displaced contents, on becoming redistributed, pass back through the opening in the partly formed cell wall provided this opening were not too small.

Cells often divide normally before the contents become redistributed, especially in Cladophora and Spirogyra, thus giving rise to two daughter cells of unequal size, a smaller one appearing a deep green from the large amount of chlorophyll, and a larger one partly colorless with only a little chlorophyll. In certain cells of the plerome of *Zea Mays* and other phanerogams, the nucleolus was thrown out through the nuclear membrane into the cytoplasm. These nucleoli never re-entered the nucleus.

Other important observations were presented touching upon the dividing cell and nucleus.[4]

[3] The full paper will be published in the *Bulletin of the Torrey Botanical Club.*

[4] This paper is published in the Annals of Botany for September 1899.

NEWS.

DR. E. B. COPELAND of the State Normal School, Chico, California, has been appointed instructor in botany in the University of West Virginia.

DR. HENRY C. COWLES, of the University of Chicago, has spent several weeks with a party of advanced students at Marquette, Mich., prosecuting ecological studies on the adjacent flora.

PROFESSOR P. H. ROLFS, formerly of Lake City, Florida, has accepted a position at Clemson College and Experiment Station. His post office is Clemson College, S. C.

THE SMITHSONIAN INSTITUTION is soon to appoint an assistant in cryptogamic botany, with a salary of $75 a month. The civil service examination will be held November 5 and 6.

WE LEARN FROM *Science* that Aven Nelson, botanist of the Wyoming Experiment Station, is making this summer an extended survey of Yellowstone National Park and adjacent forest reserves.

THE OFFICERS-ELECT of the Botanical Club of the A. A. A. S. are Professor F. S. Earle, of Auburn, Ala., *president;* Professor A. D. Selby, of Wooster, O., *vice president;* and Professor F. E. Lloyd, of New York, *secretary*.

MR. GEORGE T. MOORE, who has been an assistant in the cryptogamic laboratory at Harvard University, has accepted an instructorship in Dartmouth College, in charge of botany. This appointment recognizes botany at Dartmouth as a sister department in zoology.

THIS SUMMER has witnessed the establishment of another inland biological station. This one is at Winona lake, under the direction of Professor Eigenmann, with Professor D. M. Mottier in charge of botany. It is closely associated with Indiana University.

THE DEPARTMENT OF BOTANY of the Marine Biological Laboratory has had a very successful session. Dr. Davis and Mr. Moore, of the staff, Dr. True and Professor Cook spent the summer at Woods Hole, and Professors Campbell, Atkinson, MacFarlane, Mottier, Penhallow, Kraemer, and Lloyd, Drs. MacDougal, Shaw, Townsend, and Smith made shorter visits to the laboratory. Never before have so many botanists been present during the session.

"MOULDS, MILDEWS, AND MUSHROOMS," is the alliterative title of a guide to the systematic study of the fungi and mycetozoa and their literature, by Professor Lucien Marcus Underwood of Columbia, author of *Our Native Ferns and their Allies*. It is to be issued shortly by Messrs. Henry Holt & Company.

DR. WALTER S. SWINGLE has returned to this country after several months of travel abroad. He has brought with him many interesting and valuable economic plants that will be given an opportunity to make this country their home. The date palm and the truffle are, perhaps, the best-known forms whose introduction into this country would be greatly welcomed.

THE SECRETARY of Agriculture has planned "a publication which shall contain a résumé of the achievements of the United States in every branch of science as related to agriculture during the nineteenth century, for distribution at the Paris Exposition." Each of the bureaus and divisions charged with scientific work has been directed to contribute one or more articles reviewing the application to agriculture of the science with which it is concerned. The division of forestry intends to contribute a short history of forestry in the United States and also an account of the efforts of private land owners to apply the principles of forestry. The division solicits correspondence regarding every such effort in order that it may make a proper showing of the extent of this sort of work.